
PCR

2011/02/21

2011/08/22

.DNA

. DNA

DNA

PCR

DNA

DNA

PCR

L. tropica

L. tropica

:

DNA PCR

Using of the PCR method for Identification of *Leishmania tropica* in Syria

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ABSTRACT

Leishmaniasis spreads in eighty-eight countries, including the Syrian Arab Republic. This disease is caused by a protozoic parasite of the genus *Leishmania*. Twenty-two species of leishmania were reported to be pathogenic for human. The disease is presented in three clinical forms: cutaneous, mucocutaneous and visceral leishmaniasis. A set of methods for diagnosis and identifying the type of parasite have been developed in order to give appropriate treatment. These methods include isoenzyme analysis, serological and immunological methods and DNA hybridization. The polymerase chain reaction (PCR), using genomic or kinetoplastid DNA; provides an excellent tool for diagnosis and characterization of leishmania species. In this work, the two types of DNA were extracted in one step from cultured isolates taken from patients. We have used kinetoplastid DNA and specific primers to determine parasite species by PCR. These primers amplify species specific fragment. In this study, we have determined that the causative species of the cutaneous leishmaniasis in all patients was *L.tropica*.

Keywords: Cutaneous leishmaniasis, *L.tropica*, Polymerase Chain Reaction (PCR), Kinetoplastid DNA.

:

.(WHO, 2007)

.(Postigo, 2010) 2008
(Russell A., 1794)

.(2004)

Leishmania
*Flagellate**Trypanosomatidae*.(Alexander *et al.*, 1999)

(2004)

L.tropica.(Gradoni *et al.*, 1984) *L.major*

%40

Rodriguez *et al.*,)

(1994

Multilocus enzyme
Isoenzyme analysis

electrophoresis

Bin *et al.*, Aljeboori and Evans, 1980b; Aljeboori and Evans, 1980a)
 .(2010

Jaffe *et al.*,) cross-reaction
 .(Jaffe and McMahon-Pratt, 1987; 1984

DNA

Harris *et al.*, 1998) Polymerase Chain Reaction (PCR)
 (Noyes *et al.*, 1998

(Osman *et al.*, 1997; Ashford *et al.*, 1995)

genomic DNA (gDNA)

DNA

DNA

.kinetoplasted DNA (kDNA)

kinetoplaste

Minicircles

: DNA

DNA

800bp

800

Maxicircels

(Shlomai, 1994)

120

.PCR

Reale *et al.*, ; Laskay *et al.*, 1995)

.(Rodriguez *et al.*, 1994; 1999

DNA

rRNA

:

van ; de Lima *et al.*, 2010; Campino *et al.*, 2000) ssurRNA

(Eys *et al.*, 1992

el Tai *et al.*,) ITS

internal transcribed spacer rRNA

.(Schonian *et al.*, 2000; 2000

PCR
 DNA
 kDNA
 .PCR
 .
L. tropica
 : -1
 100 (Cytogen) streptomycin N.N.N penicillin
 26 /
 (Optika)
 %10 (Sigma) RPMI 1640
 (Cytogen)
 : DNA -2
 Hettich 10⁶ 1
 4+ / 4000 Universal 320R
 4+ PBS 10
) DNA
 K Tris .(MN
 (W/V) %0.1 56
 200µl 70 .SDS
 DNA / 8000 1

DNA . 20-
 : -3
 DNA
 .TBE 0.8 % (Roth)
 (PeQlab)
 6X Loading Buffer .0.5µg/ml
 (Biolabs) 2-Log (Roth)
 DNA . 30 100
 (Cleaver)
 (Cleaver) Gel documentation
 : DNA -4
 DNA
 280nm 260nm
 .T80 Spectrophotometer
 DNA
 A260/A280
 :PCR -5
 Thermocycler PCR
 .(Promega) Go Taq DNA Polymerase (Eppendorf)
 : Alpha DNA Primers
 .(Rodgers *et al.*, 1990) 13A: gtgggggaggggcttct/13B:atcccaccaaccccagtt
 .(el Tai *et al.*, 2000) LITSR:ctggatcattttccgatg/L5.8S: tgataccacttatcgcaact
 CSB1xR: attttcgcgattttcgagaacg/ CSB2xF: cgagtagcagaaactcccgttca
 .(Noyes *et al.*, 1998)
 PCR 25µl
 0.2mM MgCl₂ 1.5mM 1X
 . Taq 1.25U 25pmol (Roth) dNTPs
 30 35 PCR

60

95
PCR

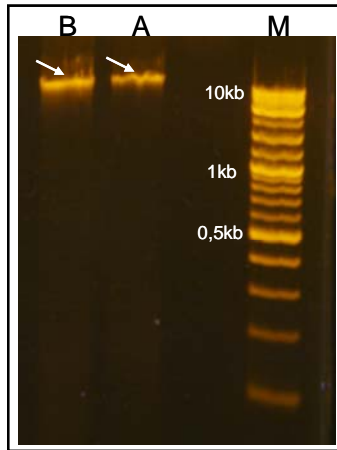
72
1.5%

-1

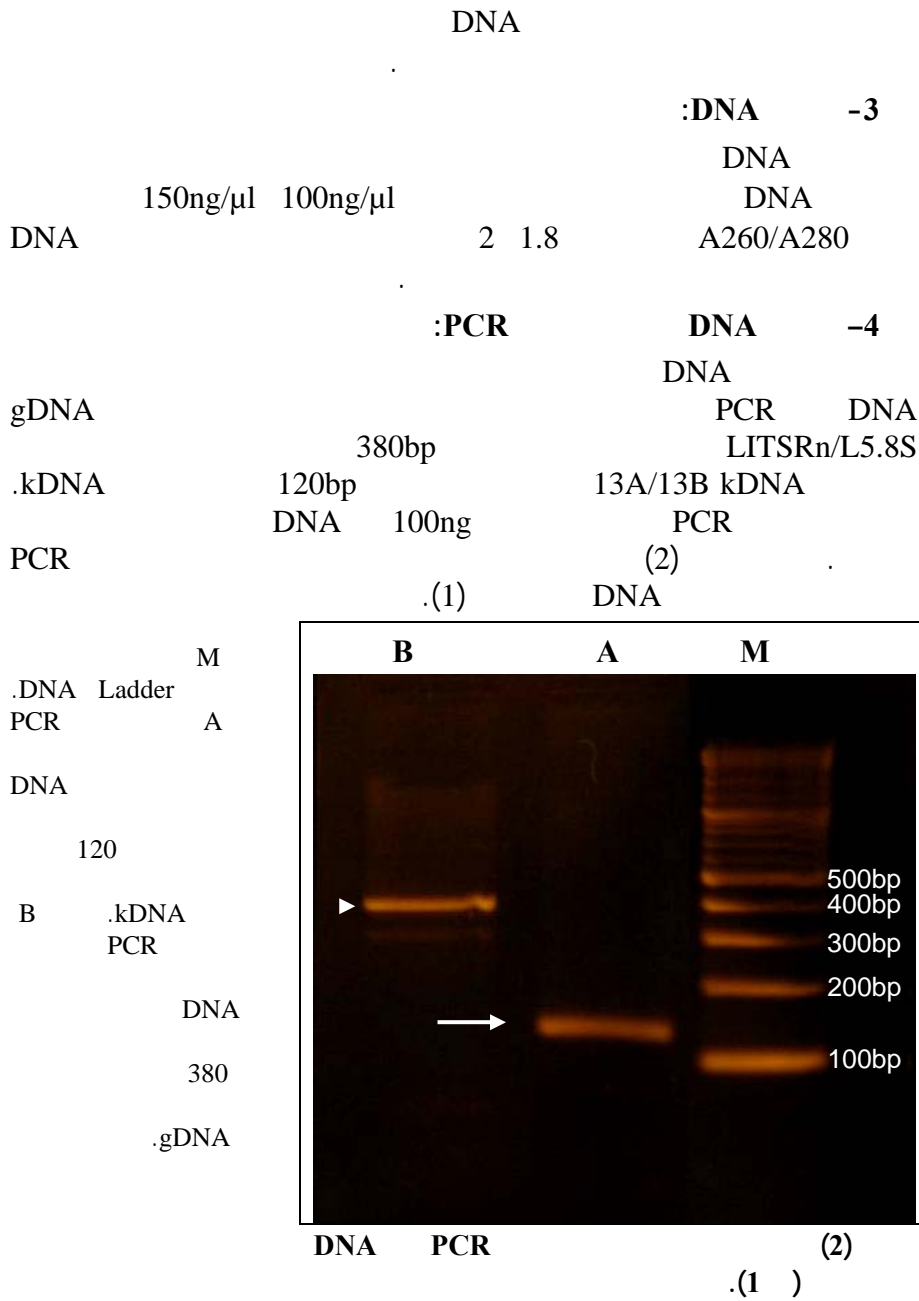
.DNA / 10^5 1 / 10^6
DNA -2

(4+) PBS
DNA
DNA
DNA
DNA
kDNA .0.8%
gDNA
(1)

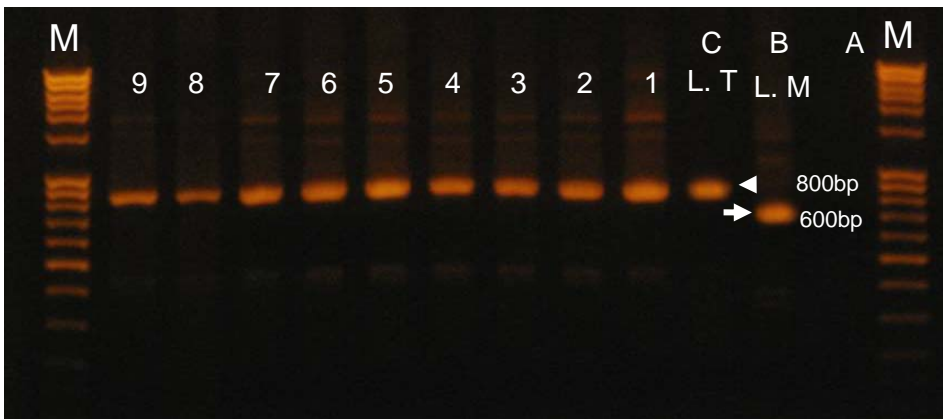
.DNA Ladder
2 1 M
DNA B A
.10kb
DNA



DNA (1)



120
DNA
380
DNA
DNA
DNA
:PCR
PCR
- 5
L.major *L.tropica*
kDNA
DNA
(CSB1XR/CSB2XF)
600
L.tropica
800
DNA PCR
L.major *L.tropica*
UMR177
PCR
(3) 1.5% PCR DNA



PCR (3)
DNA PCR A DNA Ladder M
DNA PCR B
600
L.tropica DNA PCR *L.major* C
9 1 9 1 800
DNA PCR
800
L.tropica

800

.L.tropica

PCR

DNA

PCR

.L.tropica

DNA

.RPMI-1640

DNA

; Brobey *et al.*, 2006)

(Navin *et al.*, 1992)

.(Rohousova *et al.*, 2005

(Sacks *et al.*, 1995; Magill *et al.*, 1993; Alborzi *et al.*, 2008)

; Minodier and Parola, 2007)

.(Ramanathan *et al.*, 2011

PCR DNA

.(Hajjaran *et al.*, 2011; de Queiroz *et al.*, 2011)

PCR

DNA

; da Silva *et al.*, 2004)

.(Deborggraeve *et al.*, 2008

PCR

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