

Leptospira
PCR

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(PCR)

Leptospira

Polymerase Chain Reaction

dark field

transmission electron microscope

microscope

Leptospira

(6-5.5)

DNA

PCR

(1.9-1.8)

/

Leptospira

. 16S rRNA

(331 bp fragment)

(%6.2)

(%0.45)

PCR

PCR

(%1.6)

(12) *Leptospira*

(8)

PCR

(%24)

. (%16)

Comparison of Routine Identification Methods for *Leptospira* with PCR Technique

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ABSTRACT

The study included comparison between routine methods for isolation and identification of *Leptospira* from clinical and environmental samples with PCR. Routine work included the study of morphological and cultural characteristics using dark-field microscope, transmission electron microscope and light microscope as well as some enzymatic activities. *Leptospira* showed positivity to catalase and oxidase enzymes.

Leptospira DNA was extracted and quantitated between (5.5-6) µg/µl with purity (1.8-1.9). To compare with PCR, specific primer was used for amplification of 331 bp of 16S rRNA gene.

The *Leptospira* percentage from blood and urine samples were the same (0.45% and 6.2% respectively) by using routine methods and PCR technique, while from CSF samples it gave 1.6% routinely but the PCR gave negative results. With respect to the water samples the number of isolates were 12 (24%) by routine methods but by PCR technique it gave positive result only for 8 isolates (16%).

		Leptospirosis	
Spirochaetes	Spirochaetales	Leptospiraceae	<i>Leptospira</i>
			Spirochaetes
	° 30-28		
			. (Ristow <i>et al.</i> , 2008 ; Levett <i>et al.</i> , 2006)
Phase contrast microscope		Dark field microscope	
			. (Sharma and Kalawat, 2008)
	Light microscope		Immunofluorescence
			. (Levett, 2001)
Specificity		Insensitive	

..... *Leptospira*

³ / 10⁴

. (Sharma and Kalawat, 2008)

Immunofluorescence staining

Immunoperoxidase

Leptospira

Warthin – Starry stain

. (Levett, 2001)

Immunohistochemistry

1994 1994-1985

100

0.04-0.02

1997-1992

38

234

100

0.06

2003-1998

Vijayachari and

. (Sambasiva *et al.*, 2003)

(2006) Sehgal

PCR

Leptospira

³ / 10²

(PCR quantitative)

(RT-PCR)

Leptospira

Leptospira

(MAT) Microscopic Agglutination

(ELISA) Enzyme – Linked Immunosorbent Assay Test

Leptospira

.(Gebriel *et al.*, 2006)

PCR

Leptospira

Leptospira

80

65

218

410

:

50

2007

. 2008

:

Fletcher's Medium

Dinger's Medium

Leptospira

.(Cruickshank

(EMJH) Ellinghausen , McCullough , Johnson and Harris

3-2 ° 30

et al., 1975 ; WHO, 2003)

Leptospira

EMJH

. 6-4 ° 30

:

(linear disk)

(Koneman *et al.* ,2006 ; Brooks *et al.* ,

EMJH

:

2004)

Morphological Tests

:

Light Microscope

: A

(linear disk)

. (Sambasiva *et al.* , 2003)

Dark Field Microscope

: B

Leptospira

. (Sharma and Kalawat., 2008) .

Transmission Electron Microscope (TEM)

: C

..... *Leptospira*

EMJH (6-4)

/ phosphotungstate
 . (Trueba *et al.*, 2004) /

Biochemical Tests -:

. (Koneman *et al.*, 2006)

***Leptospira* PCR** -:

Genekam *Leptospira* DNA (kit)
 Biotechnology AG (Germany)

(Scarcelli *et al.*) DNA
 (6-4) *Leptospira* *al.*, 2003)

TE ³ 1980 TE buffer DNA 20 DNA
 260 (100:1)) buffer

DNA UV-Spectrophotometer

$$\frac{50 \times 100 \times 260}{1000} = /$$

DNA / (6-5.5)
 260 *Leptospira*

280

. (1.9-1.8)

/ DNA
 16S rRNA (331 bp) /

5'- GGC GGC GCG TCT TAA ACA TG-3'
 3' - TTC CCC CCA TTG ACC AAG ATT-5'

..... *Leptospira*

. IgM-ELISA

%95.8

Leptospira

.(Green-Mckenzie ., 2005)

(5,4)

(Prescott *et al.*, 2005 ; Li *et*

Leptospira

. *al.*,2000)

Leptospira

. (Levett., 2001)

Leptospira

PCR

Leptospira

DNA

In

DNA

(331 bp)

Leptospira

vitro

DNA

.(7,6)

Leptospira

Leptospira

DNA

. *Leptospira*

(Heinemann *et al.*, 1999 ; Romero *et al.*,1998)

Leptospira

Leptospira

PCR

(Gebriel *et al.*, 2006 ; Vitale *et al.*, 2005)

PCR

(Brown *et al.*, 1995)

Leptospira

(2006) Vijayachari and Sehgal .
 (%91.4) (%95.2) PCR
 (MAT) *Leptospira*
 Microscopic agglutination test
 . DNA
 (218) *Leptospira*
 .PCR (%0.45)
 (62) *Leptospira*
 PCR (%1.6)
 16S rRNA (331 bp)
Leptospira *Leptospira*
 (80) DNA PCR
 (5) *Leptospira*
 .PCR (%6.2)
 (50)
 PCR (% 24) (12)
 . (%16) (8)
 (360) *Leptospira*
 (% 1.6)
Leptospira
 .(Levett., 2001)
 (Forbes *et al.*, 2002)
 .
 . (Sambasiva *et al.*, 2003)
 .(Plank and Deborah., 2000)
 (% 16) (50)
 (Trueba *et al.*,

Biofilm *Leptospira*

2004)

. (Ristow *et al.*, 2008)

(2004)

Nascimento

Leptospira : 1

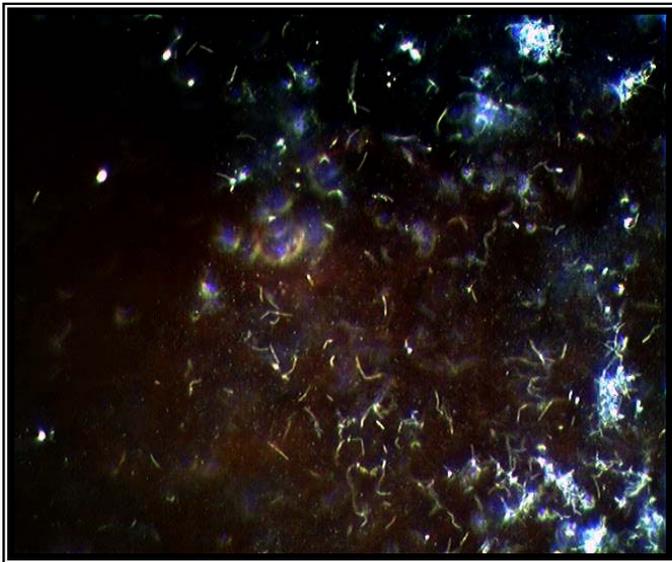
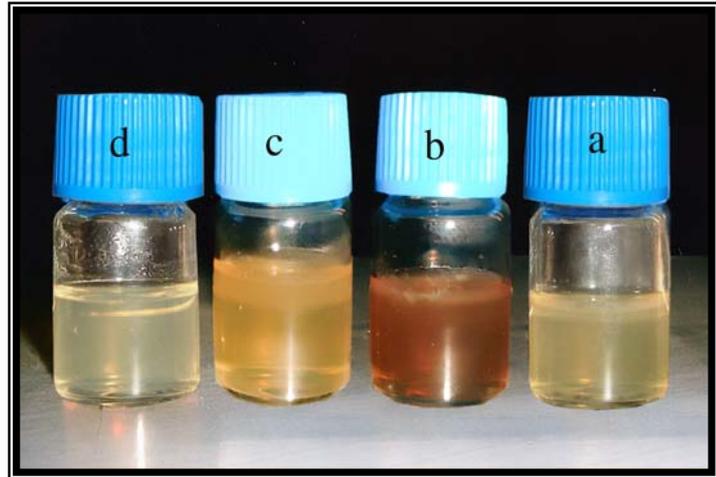
EMJH

EMJH : a

EMJH : b

EMJH : c

. () : d



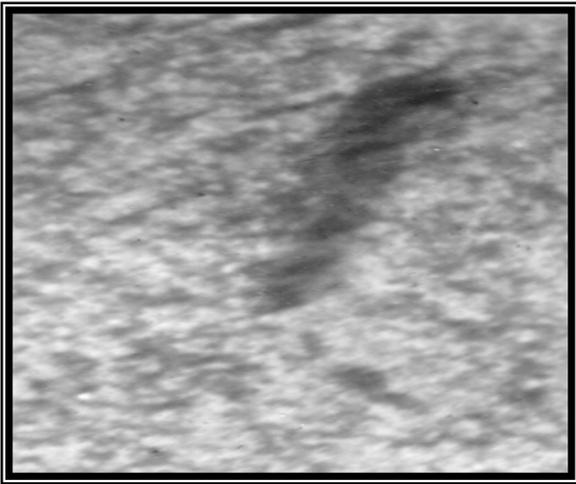
Leptospira : 3

.(400X)



Leptospira : 2

(1000X)



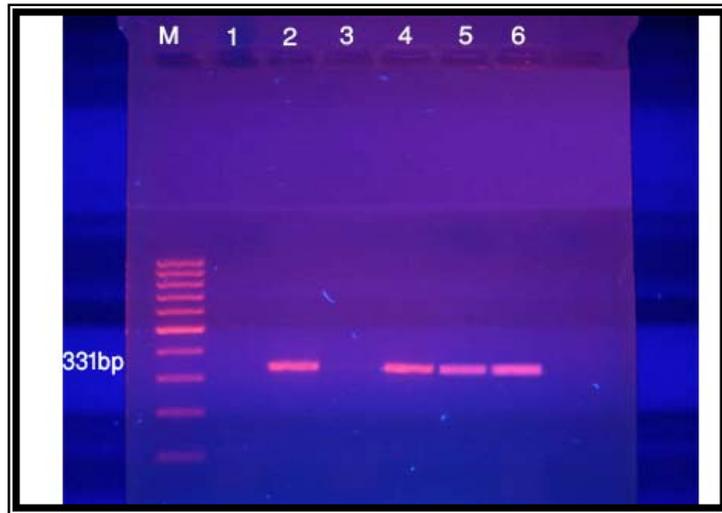
Leptospira : 5

.(17000X)



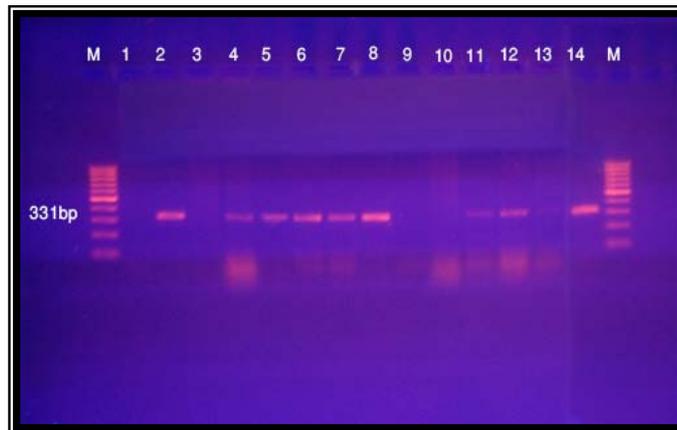
Leptospira : 4

.(10500X)



Leptospira DNA : 6

- . -1
- . -2
- . CSF -3
- . -4
- . -5
- . -6
- . -M



Leptospira DNA : 7

-1

-2

. (14,12,11,8,7,6,5,4)

. (13,10,9,3)

-M

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