

16s rDNA

nikaeen@hlth.mui.ac.ir

16S rDNA

PCR

(PCR)

Nested PCR

(LEG448-JRP) (LEG225-LEG858) ; (LEG448-LEG858)

LEG448-JRP

LEG225-LEG858

LEG448-LEG858

LEG225-LEG858

DNA

Nested PCR

PCR

promega ,Wizard® Genomic DNA Purification Kit, Madison, USA (Promega

DNA PCR PCR

DNA mL

16s rRNA R₁ Eubac27F

DNA Nested PCR

PCR μL DNA

dNTP 1X; μM

Taq DNA / μM DNA μL polymerase

$$n=z^2s^2/d^2$$

°C

(PBS)

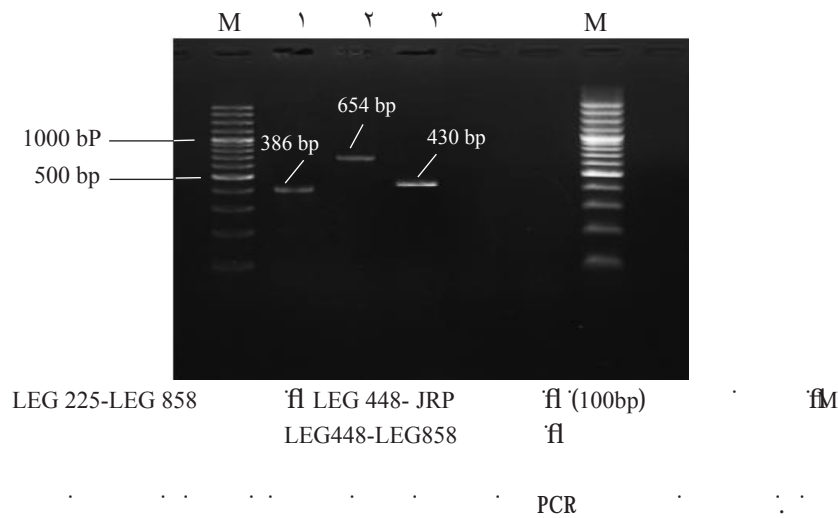
freez-thaw

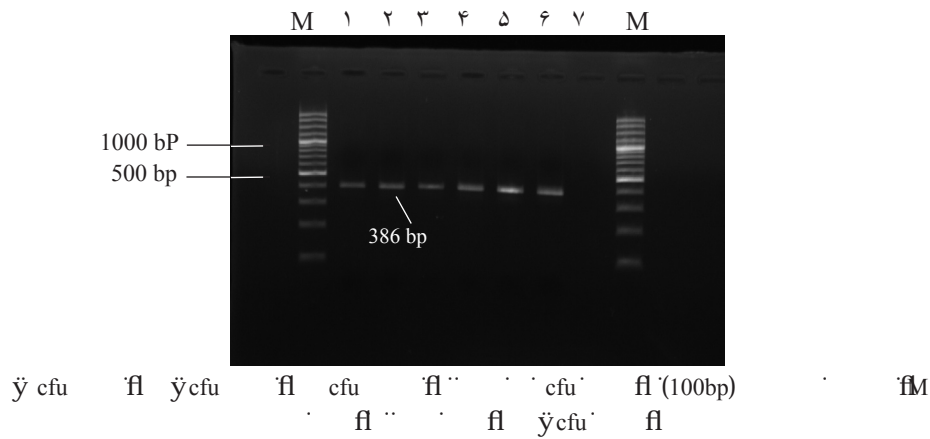
سایز محصولات (bp) PCR	ژن شناسایی	توالی پرایمرها	پرایمرها
حدود ۱۴۲۰ bp	16S rRNA	5'-AGA-GTT-TGA-TCC-TGG-CTC-A-<G>-3'	Eubac27 F 1429 R1
۶۵۴ bp	16S rRNA	5'-AAG-ATT-AGC-CTG-CGT-CCG-A-<T>-3'	LEG 225 LEG 858
۴۳۰ bp	16S rRNA	5'- AGG-GGT-TGA-TAG-GTT-AAG-AG-<C> -3'	LEG 448 LEG 858
۳۸۶bp	16S rRNA	5'- AGG-GGT-TGA-TAG-GTT-AAG-AG-<C> -3'	LEG 448 LEG JRP

PCR

تعداد مرحله و سیکل ها	تقسیمات فرعی هر مرحله	درجه حرارت	زمان
مرحله اول (۱ سیکل)	Pre- Denaturation	۹۵°C	۵min
	Denaturation	۹۴°C	۴۵s
مرحله دوم (۳۰ سیکل)	Annealing	۵۵°C	۱min
	Extention	۷۲°C	۱/ ۳۰min
مرحله سوم (۱ سیکل)	Final Extention	۷۲°C	۵min
مرحله چهارم (۱ سیکل)	Cooling	۴ °C	۳min

Loading Buffer / DNA
 DNA (UV Tech, France)
 DNA Nested PCR PCR
 DNA Nested PCR PCR
 DNA Nested PCR PCR





LEG 448-JRP

PCR

PCR

"

PCR

- 'cfu

PCR

DNA

PCR

SPSS

PCR

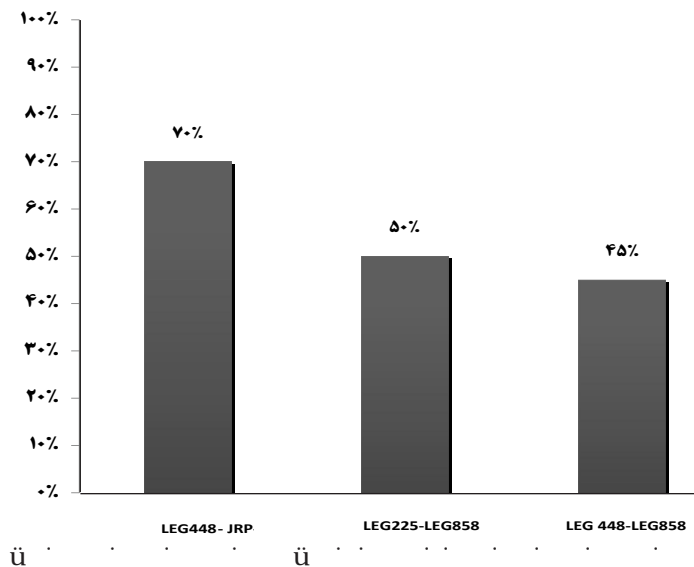
McNemar

$P < 0.05$

LEG448 -JRP

PCR

درصد فراوانی نمونه های مثبت با استفاده از پرایمرهای مختلف جهت بررسی لژیونلا



PCR

LEG225-LEG858) ، (LEG448-JRP

PCR

PCR

LEG448-LEG858

LEG225-LEG858

LEG448- JRP

JRP LEG448

McNemar

LEG448- JRP LEG225-LEG858

LEG448- JRP LEG448- LEG858

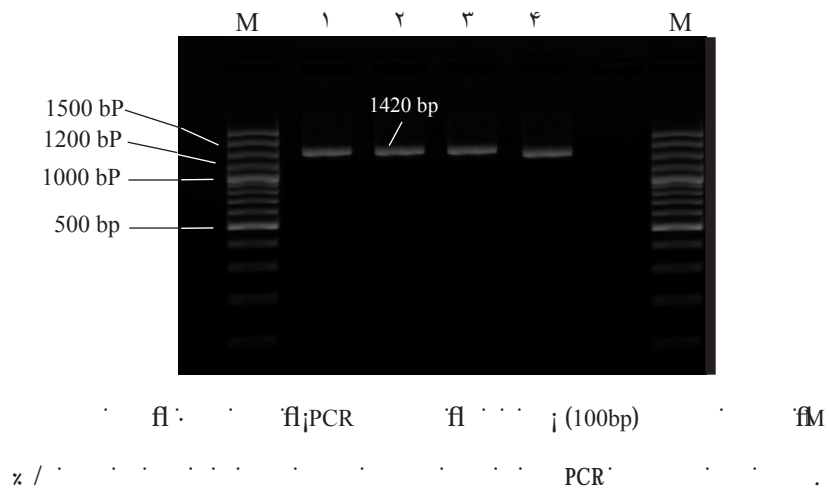
(P=)

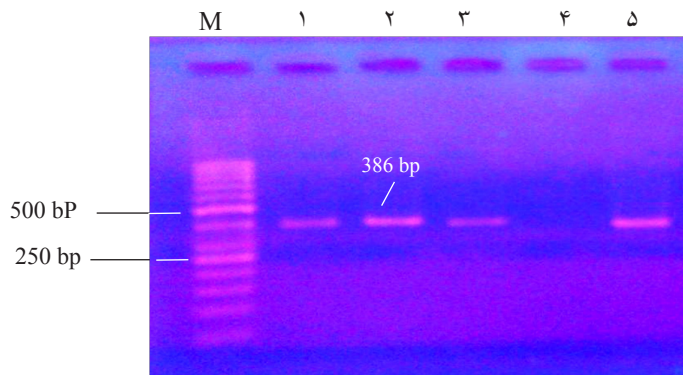
LEG448-LEG858 LEG225-LEG858

LEG448- JRP

(LEG448-LEG858 LEG225-LEG858)

PCR





M 1 2 3 4 5
500 bp
250 bp
386 bp
JRP LEG448 PCR

z /

JRP LEG448

PCR

:

sharp ness

LEG448-JRP

n

y

Nested PCR

DNA

LEG448-JRP

PCR

n iLEG225 - LEG858

"

(L

PCR

DNA

PCR

(L

PCR

PCR

1. Brooks GF, Butel JS, Morse SA. Jawetz, Melnick & Adelberg's Medical Microbiology. 23rd ed. Asia: McGraw-Hill; 2004.
2. Friedman H, Klein TW, Bendinelli M. Infectious Diseases and Substance Abuse (Infectious Agents and Pathogenesis). 1st ed. New York: Springer; 2005.
3. O'Neill E, Humphreys H. Surveillance of hospital water and primary prevention of nosocomial legionellosis: what is the evidence?. *Journal of Hospital Infection*. 2005;59(4):273-9.
4. Ishimatsu S, Miyamoto H, Hori H, Tanaka I, Yoshida S. Sampling and detection of *Legionella pneumophila* aerosols generated from an industrial cooling tower. *The Annals of Occupational Hygiene*. 2001;45(6):421-7
5. Tello R, Hill T, Hartnell G, Costello P, Stokes K. *Legionella* infected thoracic aortic graft. *Journal of Computerized Medical Imaging and Graphics*. 1993;17(1):61-7.
6. Ahmadinejad M, Shakibaie MR, Shams K, Khalili M. Detection of *Legionella pneumophila* in cooling water systems of hospitals and nursing homes of Kerman city, Iran by Semi- Nested PCR. *International Journal of Biological and Life Sciences*. 2011;7(2):70-3.
7. Devos L, Clymans K, Boon N, Verstraete W. Evaluation of nested PCR assays for the detection of *Legionella pneumophila* in a wide range of aquatic samples. *Journal of Applied Microbiology*. 2005;99(4): 916-25.
8. Ng DL, Koh BB, Tay L, Heng BH. Comparison of polymerase chain reaction and conventional culture for the detection of *Legionellae* in cooling tower waters in Singapore. *Letters in Applied Microbiology*. 1997;24(3):214-6.
9. Joly P, Falconnet PA, Andre J, Weill N, Reyrolle M, Vandenesch F, et al. Quantitative real-time *Legionella* PCR for environmental water samples: Data interpretation. *Applied and Environmental Microbiology*. 2006;72(4):2801-8.
10. Pepper IL, Gerba CP. *Environmental Microbiology: A Laboratory Manual*. 2nd ed. Amsterdam: Elsevier Academic Press; 2005.
11. Yasmon A, Yusmaniar, Karuniawati A, Bela B. Simultaneous detection of *Legionella* species and

- Legionella pneumophila by duplex PCR (dPCR) assay in cooling tower water samples from Jakarta, Indonesia. Medical Journal Indonesia. 2010;19(4):223-7.
12. Wellinghausen N, Frost C, Marre R. Detection of Legionellae in hospital water samples by Quantitative real-time LightCycler PCR. Applied and Environmental Microbiology. 2001;67(9):3985-93.
13. Declerck P, Verelst L, Duvivier L, Van Damme A, Ollevier F. PCR as a test for the presence or absence of Legionella in (cooling) water. Water Science and Technology. 2003;47(3):103-7.
14. Dusserre E, Ginevra C, Hallier-Soulier S, Vandenesch F, Festoc G, Etienne J, et al. A PCR-based method for monitoring Legionella pneumophila in water samples detects viable but noncultivable Legionellae That can recover their cultivability. Applied and Environmental Microbiology. 2008;74(15):4817-24.
15. Ko KS, Hong SK, Lee KH, Lee HK, Park MY, Miyamoto H, et al. Detection and identification of Legionella pneumophila by PCR-restriction fragment length polymorphism analysis of the RNA polymerase gene. Journal of Microbiological Methods. 2003;54(3):325-37.
16. Delgado-Viscogliosi P, Solignac L, Delattre J-M. Viability PCR, a culture-independent method for rapid and selective quantification of viable Legionella pneumophila cells in environmental water samples. Applied and Environmental Microbiology. 2009;75(11):3502-12.
17. Edagawa A, Kimura A, Doi H, Tanaka H, Tomioka K, Sakabe K, et al. Detection of culturable and nonculturable Legionella species from hot water systems of public buildings in Japan. Journal of Applied Microbiology. 2008;105(6):2104-14.
18. Villari P, Motti E, Farullo C, Torre I. Comparison of conventional culture and PCR methods for the detection of Legionella pneumophila in water. Letters in Applied Microbiology. 1998;27(2):106-10.
19. Wullings BA, Kooij VD. Occurrence and genetic diversity of uncultured Legionella spp. in drinking water treated at temperatures below 15°C. Applied and Environmental Microbiology. 2006;72(1):157-66.
20. Lin YE, Stout JE, Yu VL. Prevention of hospital-acquired legionellosis. Current Opinion in Infectious Diseases. 2011;24(4):350-6.
21. Yáñez MA, Barberá VM, Catalán V. Validation of a new seminested PCR-based detection method pneumophila. Journal of Microbiological Methods. 2007;70(1):214-7
22. Fittipaldi M, Codony F, Morato J. Comparison of conventional culture and real-time quantitative PCR using SYBR Green for detection of Legionella pneumophila in water samples. Water Sanitation. 2010;36(4):417-24.
23. Perola O, Kauppinen J, Kusnetsov J, Kärkkäinen UM, Lück PC, Katila ML. Persistent Legionella pneumophila colonization of a hospital water supply: Efficacy of control methods and Amolecular epidemiological analysis. Acta pathologica, microbiologica, et immunologica Scandinavica. 2005;113(1):45-53.

Sensitivity Comparison of Different 16s rDNA- Specific Primers for Detection of Legionella Species in Aquatic Samples

Farzaneh Baghal Asghari¹, Mahnaz Nikaeen²

¹Department of Environmental Health Engineering, Faculty of Nursing and Health, Urmie University of Medical Sciences, West Azarbaijan, Iran

²Department of Environmental Health Engineering, School of Health, Isfahan University of Medical Sciences, Isfahan, Iran

Received; 05 April 2012 Accepted; 27 July 2012

ABSTRACT

Background and Objectives: Legionella are gram-negative bacteria widely dispersed in natural and man-made water sources. Some Legionella species are pathogenic and could cause respiratory infections. Cultivation technique is the conventional method for the detection of Legionella spp. in aquatic samples. However, the method has low sensitivity and require prolonged incubation period. Therefore, Polymerase chain reaction (PCR) as a rapid method with extreme sensitivity is used. The present study was designed to evaluate the feasibility and sensitivity of PCR method for detection of Legionellas pp. in aquatic samples using three sets of primers.

Materials and Methods: In this study, 60 water samples were investigated for the presence of Legionella species using Nested- PCR technique. The sensitivity of this technique was evaluated for the detection of Legionella species in aquatic samples using three primer sets, including (LEG225-LEG858), (LEG448-LEG858), and (LEG448-JRP).

Results: The nested PCR assay revealed that detection percentage of Legionella in samples was 70 when LEG448-JRP primers were used, whereas this percentage reduced to 50 and 45 when we applied prime sets of LEG225-LEG858 and LEG448 - LEG858, respectively.

Conclusion: The results of the study showed that contamination of aquatic samples to the Legionella spp. could be easily and rapidly detected by nested PCR. However, selecting appropriate method for DNA extraction and choosing the primers are important factors in efficiency and sensitivity of detection method.

Keywords: PCR, Water, Detection, Legionella

*Corresponding Author: nikaeen@hlth.mui.ac.ir

Tel: +98 311 7922660, Fax: +98 311 6682509