[illegible]

16s rDNA [illegible]

[illegible]

[illegible] nikaeen@hlth.mui.ac.ir

[illegible]

[illegible]

[illegible] PCR [illegible] (PCR) [illegible]

[illegible] Nested PCR [illegible] (LEG448-LEG858) [illegible] (LEG225- LEG858) [illegible] (LEG448-JRP) [illegible]

[illegible] LEG448-JRP [illegible] LEG225- LEG858 [illegible] LEG448-LEG858 [illegible]

[illegible] DNA [illegible] Nested PCR [illegible]

[illegible] **PCR** [illegible]

[illegible]

Ǵǫɣ Æǫ

Æλd ŭλKŭǫ ȝY Ǵt yŭǫsǵ ÆÃŭǫ ǫɛū ÆæKǵåɛǫtKλ KǵÊŭNæŤǩ KǵåɛǫtKλ ǰæł .yŭŭyŭtłɛř Æüǿsp ȝNĂ Ǵλ ȝKsŭH ƠƟKs p ÆüǿŭĂ YłNǫ ǔȝNūKŭNū pH p Θȝłɛƒ ǴΣȝY Kλ åKǵkdȝY KÆλ ÆæKŭHNơ åKǵQǫsǿs ȝY YNΣNǫ kd ɛł p ǔyŭȝłY łȝ ȝŤǿsgł p åzŭǫ .(Ĥ)yŭł dyŭǁ åɛKsłyΣ dyŭŭtŦlƟ åKǵƧɛλ p ɛǿǁ kd ǔǴæNĤơ p dyNλ łɛåȝKȝǿλ ȝKsŭH åłɛλ åɛǫtKλ ǰæł åKǵǴŭNū ɛł ÆNJüλ ŬNŭ pY KǵÊŭNæŤǩ .yæNǁ ÆsÃlơ åKǵOŭNÃǔ Ǵλ ɛ ǫlǫ yŭŭHNǿÆǫ ɛŭNæŤǩ åȝKȝǿλ ÆŬæ yŭæKȝŭÆǫ YKǫæł łȝ ÆŬǿŭǿǫt Kǽǫsǫ åȝKȝǿλ åɛŬæY p Ơsł ȝkūȝł yŭš åɛǫūȝY Kλ yæyǁ åȝKȝǿλ Ŧæ Ǵt Ǵǿǁ dyŭNǁYpyŤǫ YNƟ Ǵλ YNƟ åȝKȝǿλ ÆǔNŭ Ǵt t’KǿǫŭNř ł ơ p ɛŭNæŤǩ åȝKȝǿλ ÆǫDžł ŘǫKǔ ÊǿəNǫNŭř ÊŭNæŤǩ .Ơsłȝŭèǽλd ÆŭNǫNŭř ŬNǿǁ ğKŭŭY Ǵλ ĤĬĬĪ ğKs ȝY ȝKλ ǰǿpł Ût’KǿǫŭNř ł ơ ŘǫKǔ łNǵ dzȝ ɛł kpɛŬǿǫ ğKǽǫŭł .yǁ p ȝłȝū ÊǿÃłYÊǿə ȝY YKf ÃæɛÃɛł åɛǫgKλ Ǵt ΘȝNDž ǰæł Ǵλ .Ơsł åȝKȝǿλ ğKǽǫŭł ÆǫDžł ȝKsŭH ÆsÃlơ dzKŬǫsY ȝY åɛǫgKλ ŘǫKf kd ȝæȝ Θłȝz ãKł dǫsł .(ĥ-ħ)YNǁÆǫ ÆūYNλd Ǵλ ɛlǫ p dyǁ ǰæȝŬæKΣ DŽÆŭ Ǵλ Êǫŭǫ ! KUǁł ǰǿŢȝǵ p YKǫūǫ ǔdzYȝNUǐKs ǔɛǿr YłɛəłȝKł dyλ ÆŰKəY Qǫsǿs ǨǿY ɛg Ǵλ Ǵt ÆŭȝKȝǿλ ɛǿŭ p Æŭȝæłŭ .(Ĩ-ĩ)yǫŭNÃǔ ŬNŭ ǰæł åyΣ yæyƑơ YȝNǫ ǔdzyǁ ùŭūNJơ Ǵλ ÊŭNæŤǩ åɛǫtKλ ÆæKsKŭǁ ȝY ǰǿpȝ Æǁpȝ ɛNŭg åɛǫtKλ Ơł Ơł p pȝ ŘǫÌNŰ p KǵȝNǫtKə ɛł ÆƟɛλ YNΣp ǔKǫł .YpȝÆǫ ȝKȝǁ ɛł ǔyŭæKȝŭÆǫ YKǫæł ğÊǫƟł æǽKǫǫ p ȝłɛūȝY p dyNȝǫ dyƱǿr łȝ ŘλKå ÆłP dyNλ dyæ Ơsǵ ǰŨȝǫ KǵǪsǿKūȝł ÆƟɛλ Ǵt ǰæł ǴǪȝΣ Kæ p (Viable but non-culturable: VBNC) yǁKǫǫ Ơł åɛǿūNǫΣ ȝKǁyǁȝ ɛł ǔΣŬæY åKǵǪsǿKūȝł ÆƟɛλ YNΣp ɛH ȝY åɛǫtKλ ɛǫȝt Oŏǫǔ ɛÅKƟ Ǵλ Ǵt ǰæł ɛŬæY ǪHǫ ŘŨł ǫ .YNǁ åłɛλ ł sKǫ Æäæłɛǁ YȝłY ğKȝǫf ł ÙÆäƱǫ åKǵǴŭNȝǫ ȝY ÊǫŭNæŤǩ åKǵǴŭNū ÆƟɛλ ǰǿšǪǵ .yæKǿǫ Ǫǵłɛə åɛǫtKλ yǁȝ .(Ī-ī)yŭyǁȝ Ǵλ ȝYKå ÆŰNǫdžǫ åKǵ njƱǫ ȝY ùŭūlj ÆǪƟ p pȝ ǰæł åKǵOŭyǪyŤǫ ɛŬæy ɛł Ơł p pȝ ǰǿKŕ OǿsKs f ǴǪŬǫ ǰæł ǰǫəɛū ɛŏǫ ȝY Kλ p ãNə ŘǫÌNŰ Ǵλ ǴΣNơ Kλ .Ơsł ÆǫèNÀ ȝKǫɛ Θyǫ Kǵ ǴǫNȝǫ ɛł ÊǫŭNæŤǩ åɛKsłyΣ OƟΣ Ǵt ɛł åɛơ Ǵǫəɛł ŭKǵp pȝ dzɛǪƟǫ ÙƠsł ɛKǿǫ (Ī dŘäłyf) p 5s rRNA ȝɬ ɛł Æł Ułλ ɛǪRŬơ ɛł dyŭÃǪsł Kλ PCR ǴǪȝΣ ȝɬɛã dyŭÃǪsł YȝNǫ ÊǫŭNæŤǩ åɛǪǫtKλ ÆæKsKŭǁ OƟΣ 16s rRNA Ơsł åłǴǫəɛł ŭřǩ åKǵ p pȝ ɛł ÆŬæ PCR .(Ĭ-ĤĦ)Ơsł Ǵǫəɛū .yǫtÆǫ ÙɛÃɛλ łȝ Ơł t p pȝ åKǵOæYpyŤǫ åŭYŘæɛ yf Ko Ǵt ÊǫŭNæŤǩ åɛǪǫtKλ åłɛλ ÆŚǪoNǫə DŽǪUł ơ åKǵOsơ Ǵt ÆǫKǫɛ åKǵǰæɛŬæKΣ PCR ŘĦǫ ÆłŭNŬłŭNǫ åKǵǽǪǩKǫd ǔyǁKλ ŘŨł ǫ Kλ kZŧt OǫǫRǫ ɐæKǫǫ Ǵt ÆǫȝŭNDž ȝY p dyNλ ÆǫsKǫǫ p kNƟ PCR ɛł dyŘÃǪsł Kλ yǁKλ dyǁ p ȝłɛū Ơł t p pȝ ɛł dyŘÃǪsł åɛơ ÃǿãY ɐæKǫǫ Ù ! KƟ åKǵǴŭNū DŽǪUł ơ ÆæKǫŭNơ ŘǿλY Ǵλ .(ĤĨ)YYɛūÆǫ ǫÊŬł Ǵλ Oǫsǫ PCR ŦǿŭŨơ YKæɛ OŬɛs ÆÃǪǫUǫ ƟKūŭKãǫ ȝY Kλ ŦǿŭŨơ ǰæł ŘȝŬ Oǿsksf ǰǿŢȝǵ p (Ĥĩ) Kǵp pȝ ɛŬæY Oǫsǫ åɛǫtKλ Ǫt åKǵOǒǫŬ ÆæKsKŭǁ Ǵλ ȝYKå ǴŬǫæł Ǵλ ǴΣNơ .Ơsł dyǁ dygKł ǫ ÙOł t p pȝ ǴǪȝΣ ɛł ɛŬæY åKǵ p pȝ Ǵλ ÆūŤæp p Ơǿsksf Kλ PCR p pȝ ÙOł t p pȝ Kλ Ǵsæ̆Ǫ ȝY pWellinghausen ƟKūŭKãǫ .Ơsæ̆dzYKs p ŭæɛs p pȝ ÙYKæɛ Æsȝɛλ ÆλdåKǵGǫNȝǫ ɛł ěĬī/ĪǴt YłY ȝKł ǁ (ĥǵǵĤ) ȝłȝKŬȝg dyǁ dzYłY DŽǪUł ơ ÊǫŭNæŤǩ Ǵλ dyNλd PCR p pȝ ÃæɛÃ ɛł dyǁ KǵOł t ɤĪǵ/Ĥ ȝY njæə ÊǫŭNæŤǩ ǴūŭKãǫ ǰǿȝg ȝY Ǵt ÆŭKf ȝY ɛǿǫ (ĥǵǵī) ȝłȝKŬȝg p EdagawaƟKūŭKãǫ ȝY .(ĤĥYNλ Oǫǫ Ơł p pȝ njsNơ ǴǫNȝǫ h ÙÆsȝɛλ YȝNǫ ÆλdǴǫNȝǫ ĤHǵ ɛł p pȝ Kλ Ǵt ÆŭKf ȝY yǁyǁ dzYłY DŽǪUł ơ ÊǫŭNæŤǩ Ǵλ dyNλd p pȝ ǴǪǫŭH .(ĤĪ)yǪYNλ ÊǫŭNæŤǩ Ǵλ dyNλd ǴǫNȝǫ ĥH ÙÆȝt PCR Ǵt Ơsǵ ȝłYȝNƟɛλ ÆæKǵOæYpyŤǫ ɛł YNƟ ǴǪNǫ Ǵλ ɛǿǫ PCR ŘǫłNŰ YNΣp ğKȝǫfł Ǵλ ȝłNơÆǫ KǵOæYpyŤǫ ǰæł ǴǪȝΣ ɛł dzȝKǁł ÆäƱǫ åKǵǴŭNȝǫ Æsȝɛλ ȝY PCR ł ŭtłp dyǪȝłYɛKλ ÆÃǪǫUǫ åKǵȝΣλ ÆäƱǫ åKǵǴŭNȝǫ Æsȝɛλ ȝY dyǪȝłYɛKλ ŘǫłNŰ YNΣp Ǵλ p YɛǪȝKt ÆäƱǫ åKǵǴŭNȝǫ ȝY ÊǫŭNæŤǩ ÆλKæYȝ OƟΣ Oǿsł .(Ĥī)YYɛū DŽUł ǫ p ȝKŬǁd ÊǫŘt PCR yæłɛə Oǿsksf ȝY PCR p pȝ YɛǪȝKt ÆλKæɛȝł ȝNŏǫǫ Ǵλ ɛljKf ǴūŭKãǫ ǰæłɛKλd Ǵs ɛł dyŘÃǪsł Kλ Æλd åKǵǴŭNȝǫ ȝY ÊǫŭNæŤǩ åKǵǴŭNū ÆæKsKŭǁ .yæYɛū åɛæȝtɛÄ ÆDžŘdžǪƟH ɛŽæłɛř OÃΣ

[illegible]

[illegible] $n=z^2s^2/d^2$ [illegible] mL [illegible] (PBS) [illegible] freez-thaw [illegible] DNA [illegible].

[illegible] promega ,Wizard® Genomic [illegible] DNA [illegible] DNA Purification Kit, Madison, USA (Promega [illegible] DNA [illegible] PCR [illegible].

PCR [illegible]

[illegible] DNA [illegible] DNA [illegible] 16s rRNA [illegible] Eubac27F [illegible] R_1 [illegible] DNA [illegible] Nested PCR [illegible].

PCR [illegible] µL [illegible] DNA [illegible] µM [illegible] dNTP 1X [illegible] Taq DNA [illegible] µM [illegible] polymerase [illegible] µL [illegible] DNA [illegible].

[illegible]

پرایمرها	توالی پرایمرها	ژن شناسایی	سایز محصولات PCR (bp)
Eubac27 F 1429 R1	5´-AGA-GTT-TGA-TCC-TGG-CTC-A-<G>-3´	16S rRNA	حدود ۱۴۲۰ bp
LEG 225 LEG 858	5´-AAG-ATT-AGC-CTG-CGT-CCG-A-<T> -3´	16S rRNA	۶۵۴ bp
LEG 448 LEG 858	5´- AGG-GGT-TGA-TAG-GTT-AAG-AG-<C> -3´	16S rRNA	۴۳۰bp
LEG 448 LEG JRP	5´- AGG-GGT-TGA-TAG-GTT-AAG-AG-<C> -3´	16S rRNA	۳۸۶bp

ÊŃǂŤ ǻƙģǻƐgƙ DŽǿƯǂơ ȝŇǒņ Ǵƙ PCR ǴǫƙƐƙ :ĥ ğþyΣ

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

DNA) ƠŀŀŖǫ ğƐǪg ȝǂŃŀǕ Ǵƙ ğƙǂþ Gǂ ƙģǴŃȜ for center HPA ,FEPTU) (ƳȝǂyŀƙǪƽǂ þŇƽ Ɛǂ ǳyŀ ǴǿHơ NCTC 12821, United pneumophila.L ,infections ÆǞŀǫ ğƐǪg ȝǂŃŀǕ ǴƙƐǙǂY ğƙǂþ Gǂ þ (Kingdom, London .yŀ ǴǪəƐū ƸǒŀȝY (ƐǎǢǫ ƙƌ)

ĶƠƟY ȝY PCR ŀŀgǂþ ǻǂƐƙ ǳyŀ ǻƐƙƽǳYƙǫƌ ğŇǪȚǫ ĶƠƟY ĥ ğþyΣ ȝY ǳyǫƌ ÆǂƙǫY ǴǫƙƐƙ ƙƙ ƙģƙŇǿþƐǦǫ .ƠəƐū ȝǂƐǎ ƸǪǕǂƙƽŇǫƐơ

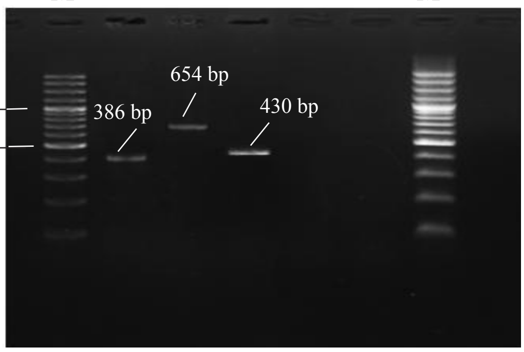
ǫƙƪŀŀ ƐȝŇəþƐǪǕǂĶȜǕǕPCRƟèŇdžȚǫÆƽȝƐƙþDŽǿƯǂơƠHΣ

Loading Buffer þěĤ/Ĩ ƐȝƙūƌğƭȝƙŧǰǂǻǂƐƙ.ƠəƐūÆǫ Ɛǂǳyŀ ƧȝƙƠğƭ ǻȝǿǫƌŪŀȝ ƠHΣþǴǪəƐūȝǂƐǎǳYƙǞǪƽǂƳȝŇǫ DNA ƠgƐƒ ǳyğƙǂǫ ǻǂƐƙ þ yǂƙǫþƐƙ ǫŇǂyǿǂ ƐȝŇəþƐǪǕǂ ǳYƙǞǪƽǂ (UV Tech, France) ƐƙƽȝƙǦŀƌ ǳƙǙǪƽY Ɛǂ ğƭ ǻþȝ ǕÆƙƌ ǻƙģǴŃȜ ȝY ÊŃǂŤ ÆǂƙƽƙŀŀþÆƽȝƐƙ Ɛǂ Ķŀǎ .yŀ þŇƽ Ɛǂ ǳyŀ ǴǿHơ DNA Ɛǂ ǳYƙǞǪƽǂ ƙ Nested PCR ĶȜǕ ǻƙģyŀƙ ǳyğƙǂǫ þ ÆƽȝƐƙ Ɛǂ yǖƙ Ǵŧ ƠəƐū ǫƙƪŀŀ Ƴȝǂyŀƙǫƽǂ ƳȝŇǫ ȝY ĀǿǢȚơ þ ÆƽȝƐƙ ǕƐȜǂƐř ǓŇŀ Ǵƽ Ɛģ ƙ ǳyŀ ĶǿǕǂơ .ƠəƐū ǫƙƪŀŀ Æƙƌ ǻƙģǴŃȜ

M ۱ ۲ ۳ M

1000 bP

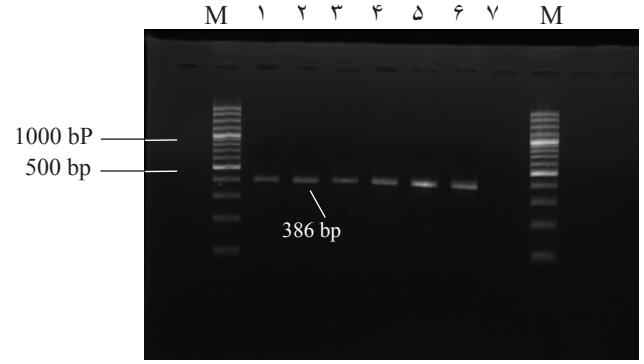
500 bp



LEG 225-LEG 858ǻƙģƐȜǂƐř (ĥ LEG 448- JRPǻƙģƐȜǂƐř (Ĥ (100bp) ÆŀŇǕǞŇǫ ƐūƙƽƙŀŀǁŀŀM LEG448-LEG858ǻƙģƐȜǂƐř (Ħ

ǜǪŀƯǫ ǻƙģƐȜǂƐř Ɛǂ ǳYƙǞǪƽǂ ƙ ÊǿəŇǫŇř ÊŃǂŤ ƳȝǂyŀƙǪƽǂ ǴŃūPCR ƟèŇdžȚǫ ƐȝŇəþƐǪǕǂ :Ĥ ĶǕŀ

سلامت و محیط دوره پنجم/ شماره سوم/ پاییز ۱۳۹۱
فصلنامه علمی پژوهشی انجمن علمی بهداشت محیط ایران



Ĥǵʰ cfuƠŏǫŮ (ħ Ĥǵ cfuƠŏǫŮ (Ħ Ĩcfu ƠŏǫŮ (ĥ ƙNǿ ʒY Ĥcfu ƠŏǫŮ(Ĥ (100bp) ÆíNŮíNǫ ƐūƘƽƘǁ (M
ÆÉÅǿǫ ǧƐǁŧ(Ī Ơǁǫ ǧƐǁŧ(ĩ Ĥǵ#cfu ƠŏǫŮ(Ĩ

LEG 448-JRP ƐŽǂƐř ɛł ŭYKÅǁƽł Ƙ PCR p ƿʒ Ơǿ5Ƙƽfǰǿūơ ƠĦΣ ɛʒNəƿƐǁŮĦ ƨǂƘǁ :ĥ ŘŮǁ

PCR p ƿʒ Ơǿ5Ƙƽfǰǿūơ

ɛł ŭYKÅǁƽł Ƙ PCR ŘŽŰ Ơǿ5Ƙƽfǰǿš Ǫǵ ÃǿÉŢơ ǰǂł ʒY Ƙ Ãlƙāǫ ʒƘŧ ǰǂł åłƐʎ.Ơəƨū ʒłƐā ÆƽʒƐʎ YʒNǫ YʒłyƳǁƽł p Ǹƽ DŽU ǂǫ Ơŏǫŭ Ƙ ÉǂƘǂƨǁŧƘ ʒǸǿƽĦĶƽ5Ǹƽ yǁʒƘə Ŧǫ YʒłyƳǁƽł ɛł Ĥ -Ĥǵ# cfu ƠŏǫŮ ɛł ÉĺƘǂƨ5 åɛƘ5Ãǿāʒ ŘŽŰ Ƙ p yǁ ǴǿHơ PCR ǀ ŀŧłp p DNA ƨłƐUǁƽł ŘŽŰ 5 Śƽ .yāYƨū ǴǿHơ ÊĦǸǂŤĺ .yǁ ǫĺ ǁ Ɛŏǁ YʒNǫ ÉǂƘǂƨǁŧƘ åĺƘgƠāʒ åpʒ ƨʎ

ĺƘgǴǁəĺƘǂ

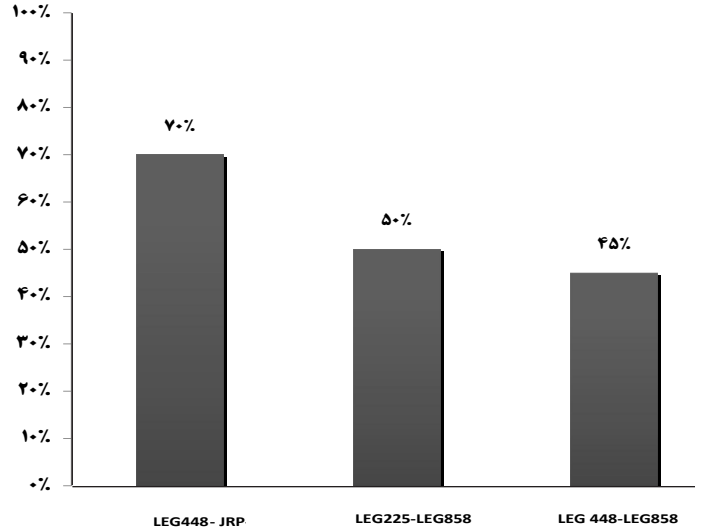
åʒĶǫd ʒǸǫɛd

ŭYKÅǁƽł ƨőĺĶǁd ɾǂĺƘǁ ƠĦΣ SPSS åʒĶǫd ǴǫĶǁƨʎ ɛł ÃǿÉŢơ ǰǂł ʒY ŮĦǸĦ ǴƽǂĺƘǫ ƠĦΣ McNemar åʒĶǫd ʒǸǫɛd ɛł .yāYƨū ɾǂĺƘǁ (P<ǵ/ǵĨ) ƨāYKÉǫ åłƨʎ .yǁ ŭYKÅǁƽł ĺƘgƨʒƐłƨř ù ǫǁUǫ .yǁ ǴǁəƐū ƨőǁ ʒY ʒłYÆǁūǫ

ĺƘgǴǁəĺƘǂ

YʒNǫ ÊǁǸǂŤĺ YǸΣp Æƽʒƨʎ ƠĦΣ ǴǁǸʒǁ ĩǵ ǴūǩĺƘāǫ ǰǂł ʒY Æƽʒƨʎ ɛł ǨǁǂZ Ǵƽ ʒǸĂ ʒłƨʒǵ .Ơəƨū ʒłƨā ǀ ĺƘǫɛd yǁĺƘ ɾǂĺƘƽ p ŮǸǁ ŭyǵĶł ǫ ƠĦΣ ŮǴǁǩĺƘāǫ YʒNǫ ÆĺƘd åĺƘgǴǁǸʒǁ åłƨʎ YʒłyƳǁƽł p Ǹƽ ɛł Ů ù ǫǁUǫ åĺƘgƨʒƐłƨř Ƙ ǴǁəĺƘǂ ŘǿŰƽł åĺƘgyǁĺƘ ŭyǵĶł ǫ p Æƽʒƨʎ ɛł yűʎ Ǵƽ yāYƨū ŭYKÅǁƽł ʒǸǒǁǫ ǰǂł ǫĶlĦ ÆĺƘd åĺƘgǴǁǸʒǁ YʒNǫ ʒY Æƽʒƨʎ p ÃǿÉŢơ ŮǴǁəĺƘǂ ŘǿŰƽł PCR ΘèǸdžŢǫ ŭyǁ ŭyǵĶł ǫ åĺƘgyǁĺƘ ƨūʒĶƠĺ Ĥ ŘŮǁ .Ơəƨū ÊǁǸǂŤĺ YʒłyƳǁƽł p Ǹƽ ƠĦΣ ƨŽǂƐř ŮǸǁ Ǵƽ ƨg ɛł ŭYKÅǁƽł Ƙ ʒłƨā Æƽʒƨʎ YʒNǫ ɾǿǁ PCR ŘŽŰ Ơǿ5Ƙƽfǰǿūơ ƠĦΣ ŭɛʒNəƿƐǁŮĦ ɾǂĺƘǁ ĥ ŘŮǁ ʒY Ǵƽ Ơəƨū Ǵƽ LEG448 -JRP åĺƘgƨʒƐłƨř ɛł ŭYKÅǁƽł Ƙ PCR p ƿʒ

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



ÊǁǸĠŤĺ ŮƽʒƐʎ ƠĦΣ ù ǫǁUǫ ŭĶǵƐŽĠłƨř ɛł ŭYKÅǁƽł Ƙ Ơǁǂǫ åĶǵ ǴǁǸʒǁ ÆĦpłƐə yDžʒY :Ħ ŘĠǁ

ÛYfY ǯƘǂǁ ÆăŏŢǭ ǻƘǵĠǁÑǯǁ ǮY ƗǮ ÆǁƘǂYǮ Ơŏƽƙƨ ƒ ǰǂƩǁǂŏƙ ƠƽƗ ǳYǁǁ ĠǂƗǮ.

ǻƘǵƩǮǂƩř Ʃǂ ǳYƘǞǁƽƗ Ƙƙ Ơǁǁǭ ǻƘǵĠǁÑǯǁ ÆǁƥǂƩǝ yDžǮYÛĦ ǨĞǁ ǮY ƥ (LEG225-LEG858) ،(LEG448–JRP) ǜ ǫǁƱǭ (LEG448-LEG858) ƠĦƩ ÆƽƮƩƙ ÊǁÑǂŤƙ ĠǂƗǮ ǳYǁǁ ƠƽƗ. ƠĦƩ ƘǵĠǁÑǯǁ PCR ƟèÑDžŢǭ ƩǮÑƥƩǁŨǂ ħ ǨĞǁ ǮY ƩÑƥƩǁŨǂ ƨǂƘǁ Ĩ ǨŨǁ ǮY ƥ Ƙǵǻƙƨǁƭƙ ÆǭÑǯŨ DŽŏƱǂơ ǻƘǵĠǁÑǯǁ Ơǁƙǭ ǻƙǁƓƙ ÊǁÑǂŤƙ Ʃǂ ǳYƘǞǁƽƗ Ƙƙ ǻƘǵƩǮǂƩř LEG448 ƥ JRP ĠǂƗǮ ǳYǁǁ ƠƽƗ.

ƤŢƙ

ÊŏƥÑǭÑř ÊǁÑǂŤƙ Ʃǂ ÆǁǁƘǁ ǻƘǵǻƙǮƘǯŏƙ ƥ ƟÊŨǂǭ Ġƙ ĠƩÑơƘ ǯǂÑǁŨ Ġƙ ÊǁÑǂŤƙ Ġƙ ƘǵǯƘǁǁƽǮƘǯŏƙ ƙƒ ÆǜYÑƚ Ġƭ ǰǂ Ġƙ ƨǂǁ ƥ ĠǁƥǮ ǮƘǁ Ġƙ ÆǁƘǁƽǮƘǯŏƙ ƽǂÑǁǂÑǂŤƙ DŽŨǂǭ ŨǂÑǁ Ʃǂ ÆŨǂ ǻƘǵ ƥ ƥǮ ǰǂƩƘƙ ÛƠƽƗ ÆǁƘǁƽǮƘǯŏƙ ǻƘǵƠǁÑǭǭ ǳƩǂƩ ǮY ƥ (ĥǵ)ƠƨǂǮƥƩǁǰ ǨǭƗÑǭ ǰǂƗ ǔǂƩƽ ƥ ǞǁǂY ÆǂƘƽƙǁƘ.

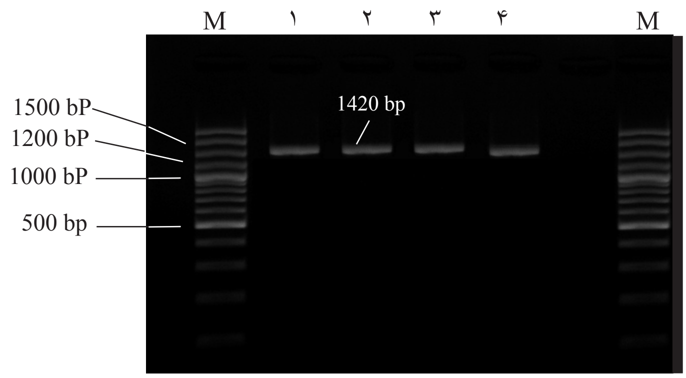
ƠƽÊǁÑǂŤƙ ǻƩǁƭƙ ÆǂƘƽƙǁƘ ǮY ǂ ƽƘǁǭ ǻƘǵ ƥ ƥǮ Ʃǂ ÆŨǂ PCR Æǂèƙ Ơŏƽƙƨ ƒ Ʃǂ ƩŨǂY ǻƘǵ ƥ ƥǮ Ġƙ ƠǁƨǁƓǁ ƥ ƥǮ ǰǂ Ǫǵ Û(ĥĤ) ƠƽƗ ÆƙǂǁƽY ǨƙƘ ǔǂƩƽ ǯǁ ƨǂƘǁ ƥ ǳYÑƙ ǮƗYǮÑƟƩƙ ǮY ÊǁÑǂŤƙ ǻƩǁƭƙ ÆǂƘƽƙǁƘ ǻƗƩƙ ƥ ƥǮ ǰǂ Ʃǂ ǳYƘǞǁƽƗ ǰŏš °(ĥĥ)ƠƽƗ ĠƥƩDž Ġƙ ǯƥƩǄǭ ƩŏǔŏƽƥƩ2 ƘŏǄǭ ǮY ÆăŏŢǭ ǻƘǵĠǁÑǯǁ ǫƘĨǁ ǌǂƩǁ ǯYƩƭ ĠǁŏĦƙ ƘǵyǁÆǜƽ ĠǜƘăǭ ǰǂ ǮY ǰǂƩƘƙ

ƨǂƘǁ ƠăY Û ƗƽƘǭ ǻƘǵƩǯǂƩř ƘƘƱǁǁ ĠǭǯƩ Ʃǂ PCR ǁ ǁƗǂƥ ǮY ƩǯǂƩř ǰǂƩƠ ƗƽƘǭ ƥ ǳYfY ǮƗƩǂ ÆƽǮƩƙ YǮÑǭ ƗǮ Ƙǵ ǁ ǂƘǭƩǝ .YÑǁ ǳYfY DŽŏƱǂơ ÊǁÑǂŤƙ ǻƩǁƭƙ ÆǂƘƽƙǁƘ ! ÑdžƠ

Ƙƙ ÆǁǝǝǁƘǵĠǁÑǯǁ ǮY ÊǁÑǂŤƙ ÆǂƘƽƙǁƘ ƠĦƩ ĠŨƘăǭ ǰǂ ǮY ǳYƘǞǁƽƗ YǮÑǭ ǜǫǁƱǭ ƩǯǂƩř ǓÑǁ Ġƽ ÛPCR ƥ ƥǮ Ʃǂ ǳYƘǞǁƽƗ Ʃǂ ǳYƘǞǁƽƗ ƟǮÑDž ǮY ĠǂYfY ǯƘǂǁ ĠǫDžƘƒ ƨǂƘǁ .ƠƥƩū ǮƗƩǂ ƟǮÑDž ǮY ƥ ƘǵĠǁÑǯǁ ëħĨ ¡LEG448-LEG858 ǻƘǵƩǯǂƩř Æǁƥ ÛƘǵĠǁÑǯǁ ëĨǵ¡LEG225-LEG858 ǻƘǵƩǯǂƩř Ʃǂ ǳYƘǞǁƽƗ yDžǮY ÛLEG448– JRP ǻƘǵƩǯǂƩř Ʃǂ ǳYƘǞǁƽƗ ƟǮÑDž ǮY ƥ ǮƗƨū ÊǁÑǂŤƙ Ġƙ ǳYÑƙ ÛYÑƙ ëĨǵYƥyƒ Ġǂ ƘǵĠǁÑǯǁ Ʃǂ ǻƩǁǂŏƙ .yǁyǁ

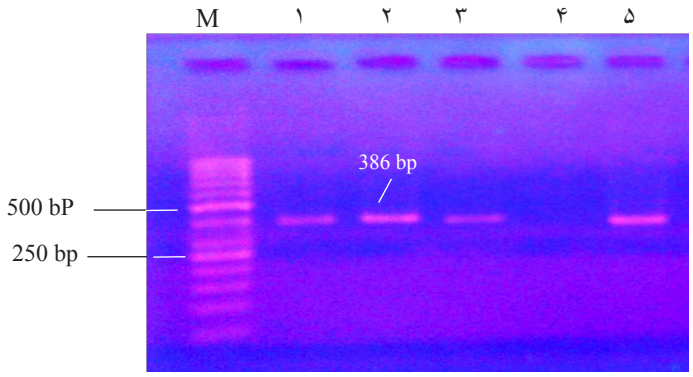
ǻǮƘǭ ǯÑǭƩǝ 2 ƘƽƗ Ʃƙ ƘǵƩǯǂƩř ǜǫǁƱǭ ǓÑǁ ĠƨǂƘĒǭ YǮÑǭ ǮY ǻƘǵƩǯǂƩř Ʃǂ ǳYƘǞǁƽƗ ƟǮÑDž ǮY ǻǮYÆǁūǭ ÙÊǁƠƗ ÛMcNemar ǻƘǵƩǯǂƩř ǰŏŢǯǵ ƥ LEG448– JRP ƥ LEG225-LEG858 yǁ ǳyǵƘǂǭ LEG448– JRP ƥ LEG448- LEG858 Ʃǂ ǳYƘǞǁƽƗ ƟǮÑDž ǮY ǻǮYÆǁūǭ ÙÊǁƠƗ Æǁƥ Û(P<ǵ/ǵǵĥ) LEG448-LEG858 ƥ LEG225-LEG858 ǻƘǵƩǯǂƩř (P=ǵ/ĥĨ) yǂYƩŨǁ ǳyǵƘǂǭ.

DŽŨǂǭ ĠǫƩū ǫƘǁǁ ǻƘǵ ǁ ǂƘǭƩǝ ƨǂƘǁ Ġƙ ĠƩÑơ Ƙƙ ǰǂƩƘƙ ǻƘǵƩǯǂƩř ƩŨǂY Ġƙ ƠǁƨǁƓǁ LEG448– JRP ƩǯǂƩř Ġǂ yǂYƩū (LEG448-LEG858 ƥ LEG225-LEG858) ǳYƘǞǁƽƗ YǮÑǭ Ġƙ Æǁƥ ǻƘǵĠǁÑǯǁ ǮY ÊǁÑǂŤƙ ǻƩǁƭƙ ÆǂƘƽƙǁƘ ǮY ƩǁÑǭ ǻƩǯǂƩř .YƥǮÆǭ ǮƘǯǁ



ÆĀǭ ǵƩǁŧ(Ĩ ÛƠǁƙǭ ǵƩǁŧ (ħ¡PCRƟèÑDžŢǭ (Ħƥ ĥ ƥ Ĥ ¡ (100bp) ÆǁÑŨǁÑǭ ƩŨƘƽƙǁƘ (M

ȟ/Ĩ ƩǮƘūǝ ǵţ ǻƥǮ Ʃƙ Ƙǵǻƙƨǁƭƙ ÆǭÑǯŨ DŽŏƱǂơ ƠĦƩ ƘǵĠǁÑǯǁ PCR ƟèÑDžŢǭ ƩǮÑƥƩǁŨǂ :ħ ǨŨǁ



ØÙÙÒ ğÇÙÙt(Ï ÛÆÅÒ ğÇÙÙt (ħ¡PCRΘèÑdžÞÒ (ĦÞ ĥ Þ Ĥ ¡(50bp) ÆÌÑÛÌÑÒ ÇūĸsĸÛÙ (M

xĤ/ ÎɛÞʒĸūd ğţ åÞʒ ÇÌ **JRPÞ LEG448**åĸgÇŽǂÇř ĸ ÊÙÑǂŤĸ ÆsʒÇ ØĦΣ ĸgĠÙÑŽÙ PCR ΘèÑdžÞÒ ɛʒÑə ÞÇÙÛĦ :ÎĶÛ Ⅱ

ŬÑÙ Ġs Ɛg Þ yfÙ Ⅱ łyÙ åɛʒĸÌ ΘÞĸÅơ (sharp ness) ţÑljÞ Þ ΘÞĸÅơ ŭǎĦÞ ʒY Þ ÙyŒYÑÌ ÆÙsĸÙÒ åĸgýĸÌ ʊyⅡ ʊyğĸǂÒ yĸÌ ÛʊYÑÌd ĠÙÑŽÙ ÆǂĸsĸÙ Ⅱ Þ DŽǾUǂơ ʒY ùQQUÒ åĸgÇŽǂÇř Ġ Ɛ ⅃ÙÒ Ġt Oʊł ʊYĶÅQsł YʒÑÒ åĸgÇŽǂÇř OǾsĸsƒ Ġ NjÑÆÒ .YYƐūÆÒ ʊYÑÌd ĠÙÑŽÙ ÆǂĸsĸÙ Ⅱ ǫyŰ Kǂ Þ ÆǂĸsĸÙ Ⅱ

ΟĦΣ ĠQǝʒ ʒĸt Ġ Þ Þʒ Ġt YĦY ŽĸǂⅡ ǰǾšš ǪǧĠüĶĸăǫ ǰǂł sǂĸQ NjÑÆÒ sǂĸQ Ġt åłĠÙÑūu Ġ ʊYÑ ÆⅡsĸÙÒ Þ Þʒ DNA ƧłƐUQsł ΟⅡÙÒ ĸgĠÙÑŽÙ ǫĸŽơ ʒY ĸgåƐQ⁞tĸ ÆǫÑŽŰ åĸgÇŽǂÇř ĸ PCR Ġ .yⅡ Þ ʒłƨū

åƐǾū Ġ⅃ǾQ

Þ Þʒ kĸUQ⁞Ħ ĸ Ġt YĦY ŽĸǂⅡ Ɛljĸƒ ĠüĶĸăǫ ɛł ŘDžĸƒ sǂĸQ

ł sĸÙÒ åĸgÇŽǂÇř kĸUQ⁞Ħ Þ DNA ƧłƐUQsł ΟĦΣ ł sĸÙÒ łʒ ÊÙÑǂŤĸ Ġ ÆăǾŢǫ åĸgĠÙÑŽÙ ÆūYÑÌd ÆūYĸs Ġ ŽłÑơÆǫ ŘŽŰ OŰƐs Þ ÆūŤǂÞ ÛOǾsĸsƒ .YĦY DŽǾUǂơ OŰƐs Ġ 2 ĸsł Ç Ġt Oʊł PCR åĸǂłƨǫ ɛł ÛsǂĸQ Ġ ÆĸKǾQsY ʒY èĸ ɛł åƐǾūÑǪΣ ΟĦΣ łʒ ł sĸÙÒ ÆÌƐQÙt ΘĸǫłyƋł ŽÑơÆǫ ŽƋ ! ĸƱ åĸgnjǾŢǫ ʒY ʊŤǂÞ Ġ åƐQ⁞tĸ ǰǂł ɛł ŘDžĸƒ åĸgOⅡÑÅÛ .YÑŽÙ łƐΣł ĠǪDžĸəÊ

ɛł ʊYĶÅQsł ΘʒÑDž ʒY ÊÙÑǂŤĸ Ġ ʊYÑÌd OⅡÙÒ åĸgĠÙÑŽÙ YłyfÙơ ƐQ⁞ǂǾ ÆǪǾƱ ĸgĠŽǂÇř ƐŰǂY Ġ OⅡsⅡ LEG448-JRP ŽǂÇř åƐQ⁞tĸ ÆǂĸsĸÙ Ⅱ ʒY ŽǂÇř ǰǂł YĶǂƐ OǾsĸsƒ ŭǎĦÞ ʒY Ġt ʊYÑ ʒY Ġt åłĠüĶĸăǫ ʒY .yÙtÆǫ ŽĸǾł łʒ ÆÌd åĸgĠÙÑŽÙ ʒY ÊÙÑǂŤĸ ěĬī Ġt yǂYƐū DŽU⁞ǫ ƨǾ ĠQǝƐū ǫĸ⅃Ħ ŦǂŤǪ ʒY ĥǵǵĨ ğĸs åĸgĠŽǂÇř ɛł ʊYĶÅQsł ĸ Þ Nested PCR Þ Þʒ ĸ ĸgĠÙÑŽÙ ĸ Ġt ÆĶf ʒY Ůyfǫsǵ OⅡÙÒ ÊÙÑǂŤĸ ƐŏⅡ ɛł LEG448-JRP ĸgĠÙÑŽÙ ěĬī ¡LEG225 - LEG858åĸgĠŽǂÇř ɛł ʊYĶÅQsł .(ĥĦ)yfÙYÑÌ ʊyⅡ Þ ʒłƨū ΟⅡÙÒ

ŽĸǫƐ ʒY łʒ ùQQUÒ åĸgĠŽǂÇř ĠsǂĸÆÒ OǾŽǵł sǂĸQ ǰǂł åĸgĠÙÑŽÙ ƐQŽt ǰǾŽUơ ɛł kĸQ⁞ΣłOĦΣ ÆÌd åĸgĠÙÑŽÙ ÆsʒÇ ÆsʒÇ OĦΣ Ġt ÆơĸüĶĸăǫ ʒY .yğYÆǫ ʒłÇă yǾtĸơ YʒÑÒ ÛOⅡÙÒ ĠŽǂÇř ŬÑÙ Ŧǂ ÛPCR Þ Þʒ ɛł åƐǾūʊƐĦÌ ĸ ÆăǾŢǫ åĸgĠÙÑŽÙ OǾsĸsƒ OQŰ Ġ YʒĦY ğĸŽQ⁞fł YƐǾūÆÒ ʒłÇă ʊYĶÅQsł YʒÑÒ ʒłyÆǫ ɛł ƐQŽt ÛʊyⅡ ÆǂĸsĸÙ Ⅱ ʊYÑÌd Þ OⅡÙÒ åĸgĠÙÑŽÙ ÛǰǾĸř ʒY Qǵ ÛùQQUÒ åĸgĠŽǂÇř ɛł ʊYĶÅQsł ǰǂłƐ⁞ÌÌd .yⅡĸ ÆüāłÞ ÆǂĸsĸÙ Ⅱ Ġ Ɛ⅃ÙÒ ÛÆÌǾĸ åĸgĠÙÑŽÙ Qǵ Þ ÆăǾŢǫ åĸgĠÙÑŽÙ ʒY Ġt yⅡ yǵĦÑơ YĶǂƐ åĸgÆüŤǂÞ Þ OǾsĸsƒ ĸ ÆǂĸgĠŽǂÇř åĸăơ ʒĸšY ʊyⅡ ÆǂĸsĸÙ Ⅱ ʊYÑÌd åĸgĠÙÑŽÙ YłyfÙơ ΘʒÑDž ǰǂł .(Ī)YÑÌ yǵĦÑUⅡ åYĶǂƐ

ÆⅡĸsÛǂ njǂłƐ Ⅱ Ġt ǰǂł Ġ ĠΣÑơ ĸ ĠQǝƐū ǫĸ⅃Ħ ĠüĶĸăǫ ʒY yfÙ ÛYÑÌ Qtĸƒ Ķƒł£ǫ ǫĸŽơ ʒY PCR ⅃ łtłÞ ǫĸ⅃Ħ ΟĦΣ ĸ ʊyⅡ ʊyğĸǂÒ åĸgýĸÌ ÛPCR ΘèÑdžÞÒ ɛʒÑəÞÇÙÛĦ ɛł ʒYÑÌ DŽU⁞ǫ ŤĸŢÌ ɛł ŽǂÇř OÅΣ ŬÑÙ Ġs Ɛg ɛł ʊYĶÅQsł

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

Farzaneh Baghal Asghari[1], *Mahnaz Nikaeen[2]

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

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

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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*