Æ ł gpŤř ÆŽQŰ å ĆęKIQdžə ÛnjØŢę p OęÊs ĆQ l ę
ŽłÇæł njØŢę O‖łyFIA ÆŽQŰ ǰŽ l IH
ĥĪĥ Ko ĥĩH ΘKŢĦDž ,ÂHĬĤ ǯóæKř ,ęNs ŁŽKŽ‖ ,Q l ŁřŁŽpY

16s rDNA ĆØfKIA ÆDžKdžQ[ƠŁ ù Q[UQ å KgÇŽæłÇř OØ5KsfĆsáKÆę ÆλŁ å KgĆGIINŽIA ZY ÊIINźŤk å Ç[tKλ å KgĆGIINūÆáK5KDŁ‖ ZY

ĥ ǰÓħŁ Ŧ ÓIA ɕKDłHę ¡Ĥ å ÇŭDžł ğKÆλ ĆIHɕÇə
nikaeen@hlth.mui.ac.ir ÛnjØŢę O‖łyFIA ŁpÇū ÛO‖łyFIA ŁyĞ ł IHY ÛŽKHåDžł ÆĞ‖ Çř ęNQŰ ŁKŰ ł IHY ÛŽKHåDžł :ğNlsę ŁyIsáNI

ŤĤ/ġħ/ğĪ : p Ç*zř ŤĤ/ġĤ/ĤĪ :OaKźZY

ŁyØĞš

ŽKsI åłÇλ å Ç[tKλ ǰæł å KgĆGIINū ɛł ÆNJIJλ .yFIIyy/IłtłÇř ŽKsI OƠKs p ÆüØIIĂ ÆλŁ ŭKIQęZY Ćt yØ[sg ÆÅIęę ęÇū å KgåÇ[tKλ KgÊIINźŤk :ÙyG p ĆIIØęɛ KØIIY Ćλ Kęł YPZÆę ZKŽ‖ Ćλ ÊIINźŤk å Ç[tKλ ÆáKsKD‖ ZY ǰØpPZ Æ‖PZ O ł t p PZ .yIN‖ ÆsÅIIo å KgOIINÀÜ Ćλ Ç l ęę yIIHNoÆę p ŁYNλ łɛåZKŽØ ɛłÇŽØ?ř åłIIÇ l ę / IłtłP ŦØIUo ŁɛPÇ?ł Ůə æKØ Ćλ ÆIKØ[sY åłÇλ ɛKØ YZNę ÆIèNĂ ŽKęɛ Θyę p ǰØæKř OØsKs f ĆQŽΣ ɛł p PZ ǰæł å KgOæYPyTę ZY ÊIINźŤk ÆáKsKD‖ ZY PCR p PZ YÇ[ZKt ÆIKæÇZł ZNõIę ĆλÇ1ǰkf ĆūIKăę .YÇØūÆę ZłÇã ŁYKÅ[sł YZNę ÊIINźŤk å Ç[tKλ ÆáKsKD‖ OIIΣ (PCR) .OəÇū ęK l ę ΘPKÅ[ę ÇŽæÇř OÅΣ Ćs Kλ ÆλŁ å KgĆGIINŽI

ǰæł OØsKs f p Oə Ç ū ZłÇã ÆsZÇλ YZNę Nested PCR p PZ ɛł ŁYKÅ[sł Kλ ÊIINźŤk ZNNJf OIIΣ k ł ĆIINŽI ĩġ ĆūIKăę ǰæł ZY :ÆsZÇλ p PZ ÊIINźŤk ÆáKsKD‖ OIIΣ(LEG448-JRP) p (LEG225- LEG858) ¡ (LEG448-LEG858) ĶęK‖ ù Q[UQ ÇŽæÇř OÅΣ Ćs ɛł ŁYKÅ[sł Kλ p PZ .yæYÇū ÆIKæÇZł

Kλ Ćt ÆσZNDž ZY yFIyλ ŁYHY DŽØU ł σ ÊIINźŤk Ćλ ŁYNλł LEG448-JRP ÇŽæÇř OÅΣ ɛł ŁYKÅ[sł ΘZNDžZY KgĆGIINŽI ëĒċĆGIIKăę ǰæł ZY :KgĆG[əKæ ÊIINźŤk Ćλ ŁYNλł KgĆGIINŽI ë ħĨ ¡LEG448-LEG858 ÇŽæÇř OÅΣ ɛł ŁYKÅ[sł Kλ KgĆGIINŽI ëĨġ¡LEG225- LEG858ÇŽæÇř OÅΣ ɛł ŁYKÅ[sł .yFIyλ p ZłÇū

p PZ ĆQØsP Ćλ OØÊs Ćλ p Æ[tłZ Ćλ yFINoÆę ÊIINźŤk å KgĆGIINūλ Ćλ Æλł å KgĆGIINŽI ÆūYNλł Ćt YHY ŽKł l ÇIjKf GūIKăę ɛł ĶDžKf ɐæKIII :å Ç[ū Ć[ØIII p ÆæłZKt ZY QHę ĶęHNŰ Û ł sKQę å KgÇŽæÇř k KU[IIH p DNA ƧłÇU[sł OIIΣ ł sKQę p PZ k KU[IIH ǰæÇKλ .YNλ ŁYHY DŽØU ł σ Nested PCR .y[IIsg DŽØU ł σ p PZ Ø Øsłs f

*ÊIINźŤk ÛÆáKsKD‖ Û k ł Û**PCR** :å yØQt ŽKūţłP*

چکیده

زمینه و هدف: تشخیص سریع و دقیق عفونت‌های ناشی از لژیونلا در نمونه‌های محیطی و بالینی از اهمیت بالایی برخوردار است. هدف از این مطالعه مقایسه حساسیت پرایمرهای مختلف اختصاصی ناحیه 5s rRNA و 16s rRNA در تشخیص لژیونلا در نمونه‌های آب و بالینی با استفاده از روش PCR بود.

روش بررسی: در این مطالعه توصیفی-مقطعی، ۱۰۰ نمونه آب و ۱۰۰ نمونه بالینی (ادرار و خلط) از بیماران مشکوک به عفونت لژیونلا جمع‌آوری شد. پس از کشت و تأیید نمونه‌ها، DNA استخراج شد و با استفاده از پرایمرهای اختصاصی ناحیه 5s rRNA و 16s rRNA به روش PCR مورد بررسی قرار گرفت. حساسیت، ویژگی و ارزش‌های پیش‌بینی هر پرایمر محاسبه شد.

یافته‌ها: از ۱۰۰ نمونه آب، ۳۲ نمونه با کشت و ۴۱ نمونه با PCR مثبت شد. پرایمر 5s rRNA حساسیت ۹۳٪ و ویژگی ۹۷٪ داشت، در حالی که پرایمر 16s rRNA حساسیت ۷۸٪ و ویژگی ۹۴٪ نشان داد. در نمونه‌های بالینی، پرایمر 5s rRNA ۸۹٪ حساسیت و ۹۶٪ ویژگی داشت. ارزش پیش‌بینی مثبت و منفی پرایمر 5s rRNA به ترتیب ۹۵٪ و ۹۴٪ بود.

نتیجه‌گیری: پرایمر اختصاصی ناحیه 5s rRNA در تشخیص لژیونلا در نمونه‌های آب و بالینی حساسیت و ویژگی بالاتری نسبت به پرایمر 16s rRNA دارد و می‌تواند به عنوان روش مناسب‌تری برای پایش محیط‌های آبی و تشخیص بالینی مورد استفاده قرار گیرد.

promega ,Wizard® Genomic Ơźg ƙ DNA ƧłƐƱƖƽł DNA Purification Kit, Madison, USA (Promega Ơǽƙ ȝƴ Ǵŧ Ơəƈū ǫƘƛ ŀ ưƴƖƈƙƽ ǴĸƘƱȝƘŋƖƽƴ ĀŀĸÁ p ƿȝ ƙ ÊŀŇǽŤĭ ǻƐƖŧƘ ÁǽƙƽƘƙŀ Ơ ŀ ŠưƴǫƋ Ơ ƽƴ Ǵ DNA .Ơəƈū ȝłƐ̈ưƴǺƖƽł ƴȝŇǫ PCR

PCR p ƿȝ

ȝŇŏŀǫ Ǵ ğpł Ǵǫfƈǫ ȝƴ ÛƘǵ ǴŀŇŽŀ DNA ƧłƐƱƖƽł ɛł 5 ř DNA ƐźƘŪơ ǰźŀš Ǫǵ p ưƴȝłƴƐƘ ŘǫłŇŪ̃ ƴŇΣp ÁŀƘǽƐȝł ȝţ ɛł Á ł Uŀ ÛÊŀŇǽŤĭ ǻƐƖŧƘ ÁŀƘǽƴȝ Ơźƽƙƽ f ŀ ǽłƶəł ǻłƐŀ ǻƘǵƐŽǽłƐř ɛł ǻƐźūưƐƔŀ Ƙŀ ÛƘǵǻƐƖŧƘ 16s rRNA ÆǫŇŽŪ .(Ĥ ğpyΣ) Ơəƈū ȝłƐ̈ǻ ƐźƘŪơ ƴȝŇǫ ĤħĥĬR$_1$ p Eubac27F ÛĬĸŀã Ǵǫfƈǫ ȝƴ ǴǫəƘǽ ƐźƘŪơ DNAɛł ưƴƘǻŀƽł Ƙŀ yźŀ Ǵǫfƈǫ ȝƴ ÁĖłŇơ Ƙŀ ƵŽǽłƐř ΟǻΣ Ǵƽ ɛł ưƴƘǻŀƽł Ƙŀ Nested PCR ĬŽŪ ǫƘƛ ÊŀŇǽŤĭ ǻƐƖŧƘ DŽźƱŧơ ΟĦΣ Ĥ ğpyΣ ȝƴ ưƴŀ ɛŧƶ .(ĤĬpĪ)Ơəƈū

PCR ğŇǪŢǫ ĥĨ µL ɛł ÛǴǫfƈǫ pƴ ƈğ ȝƴ DNAƐźƘŪơ ΟĦΣ ƈğ Ùğ/ĥ µM Ơŏǫů ƙ dNTP 1X¡Ơŏǫů ƙ ƐəƘ ǻpƘf Taq DNA yƒłp Ĥ/Ĩ ¡ ǵ/ĥµM Ơŏǫů ƙ ƘǵƐžǽłƐř ɛł ǫłyŧ ȝƘƍ ȝƴ .yŀ ưƴƘǻŀƽł ŇŬĸł DNA ¡Ĥ µL ppolymerase

ÊŀŇǽŤĭ Ǻƽȝƈ Ơ ŀ ưƴƘǻŀƽł ƴȝŇǫ ǻƘǵƐžǽłƐř :Ĥ ğpyΣ

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

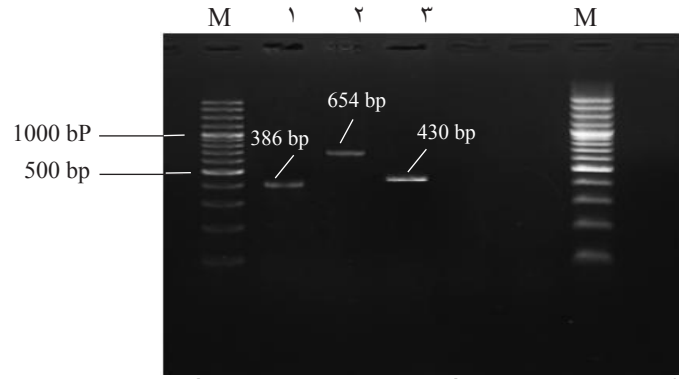
Ê‌ÌÑ¾ÎÌ å‹ÍgåÉ¦gÍ DŽÚU ǂ¤ ƷÑÖ¦Ç Ǵ PCR ǴÇÍÉÌ :ĥ ğpyΣ

تعداد مرحله وسیکل 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

DNA) OÍ¦Ç ğÉÍ¦g ƷǂÑ¦Ñ¦Í Ǵ ğ¤¾p G¾ ÍgǴÍÑƷÍ for center HPA ,FEPTU) (YƷłyÍÍÇ¦ƷǂÍ p Ñƽ ɛǂ ¤yÍ ǴǾHơ NCTC 12821, United pneumophila.L ,infections Æ¾Ä¦Ç ğÉÍ¦g ƷǂÑ¦Ñ¦Í ǴǀÉÚ¾Y ğ¤¾p G¾ p (Kingdom, London .yÍ Ǵ¦ə£ū ÉŏÍ ƷY (ÉĂ¾Ç ǩd)

ǨƟǂY ƷY PCR ǀ ¦gǂp å¾Ç¤ ¤yÍ å£¤¦¤ÇǩÍ ğÑ¦Ç¦ ǨƟǂ ĥ ğpyΣ ƷY ¤yǩÍ Æ¾ÍǩǴ ǴǩÍÉÌ Íǩ ÍǩǩÑǾoÉÉĞǾǩ .Ơə£ū Ƶ¾Ç¾ ÉǪÚ¾¤ƷÑǩÉơ

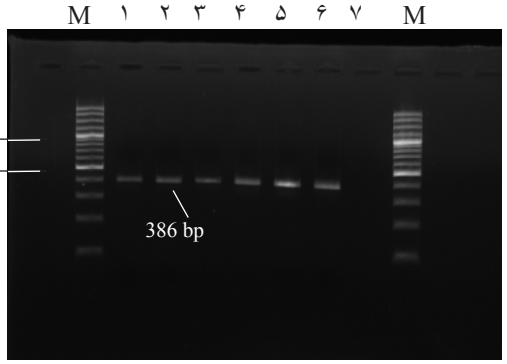
ǩÍ¦Ç¾ÑəÉǪÚǂǨƷÚPCR ΘèÑdžÇ¦ǩÆ¤ƷÉÌp DŽǾU ǂ¤ ƠǂΣ



LEG 225-LEG 858 å‹ÍgÉƷ¾ÉÍ (ĥ LEG 448- JRP å‹ÍgÉƷ¾ÉÍ (Ĥ (100bp) ÆÌÑÚÌÑ¦ ÉūÍ¤ÍÑ¦ (M
LEG448-LEG858 å‹ÍgÉƷ¾ÉÍ (Ħ

ù ǪǪǩ å‹ÍgÉƷ¾ÉÍ ɛǂ ¤yǨÍÅ¦¤ÍÍ Ê¡ÑƽÑ¦Ñ¾ Ê‌ÌÑ¾ÎÌ YƷłyÍÍÇ¦¤ÍÍ ǴÍÑūPCR ΘèÑdžÇ¦ ɛƷÑəÉǪÚǂǨ :Ĥ ǨÚ ‖

Loading Buffer þěĤ/Ĩ ɛƷ¤ūd ğţ Ƹ¤t ǰ¾ å£Ç¦.Ơə£ūÆǪ ɛ£ ǂyÍ ƧƷ¤ʊ ğţ å¿ÓǪd ŪÍƷ ƠĦΣ þ ǴǪə£ū Ƹ£Ç¤ ǂyǨÅǪ¦ƽ£ YƷÑǪ DNA Ơg£ƒ ǂy¤g¤ǂǪ å£Ç¦ þ y¾¤ǪþÇ¦ ǪÑ¾ýÓd¦ ɛ£ ɛƷÑəÞÇ¦ǪŨĦ ǂYǨÅ¦¤ƽ£ (UV Tech, France) ɛ¤ƽƷ¤ǴǪ‖d ǂ¤ŨǪ¦ƽY ɛ£ ğţ å¿Ƹ ÛÆ¦d å‹ÍgǴÍÑƷÍ ƷY ÊÌÑ¾ÎÌ Æ¾¤ƽ¤Ǫ‖ þ Æ¤ƷÇ¦ ɛ£ Ǩŭ¤ .yÍ þ Ñƽ ɛ£ ǂyÍ ǴÍÑƽ DNA ɛ£ ǂYǨÅ¦¤ƽ£ Íǩ Nested PCR ǨƷŨ å‹ÍgyÍǩ ǂy¤g¤ǂǪ þ Æ¤ƷÇ¦ ɛ£ yũ¦ Ǵ¦ Ơə£ū ǪǩǀÍ YƷłyÍÍÇ¦ƽ£ YƷÑǪ ƷY ĀÓ¾ÎƬʊ þ Æ¤ƷÇ¦ ÛÇƷ¾ÉÇŕ ŨÑÍ Ǵƽ ÇǵÍǩ ǂyÍ ǨƟÚǂ¤ƠơÆÉd å‹ÍgǴÍÑƷÍ.



Ĥǵʰ cfuƠŏǫŮ (ħ Ĥǵ cfuƠŏǫŮ (Ħ Ĩcfu ƠŏǫŮ (ĥ ƙŃŏ ʒƳ Ĥcfu ƠŏǫŮ(Ĥ (100bp) ÆĺŃŮĺŃǫ Ɛūĸƽĸŀ (M
ÆÉÅŀǫ ğƐǪŀŧ(Ī ƠǪĸǫ ğƐǪŀŧ(ĩ Ĥǵ#cfu ƠŏǫŮ(Ĩ

LEG 448-JRP ƐŽǂǂƐř ɛɬ ŀżƳĸÅǫƽɬ ĸ PCR p ƿʒ Ơǿƽĸƽ f ǰǿǘơ ƠĦΣ ɛʒŃəƿƐǪŮĸŦ ɾǂĸǪǁ :ĥ ĸŮǁ

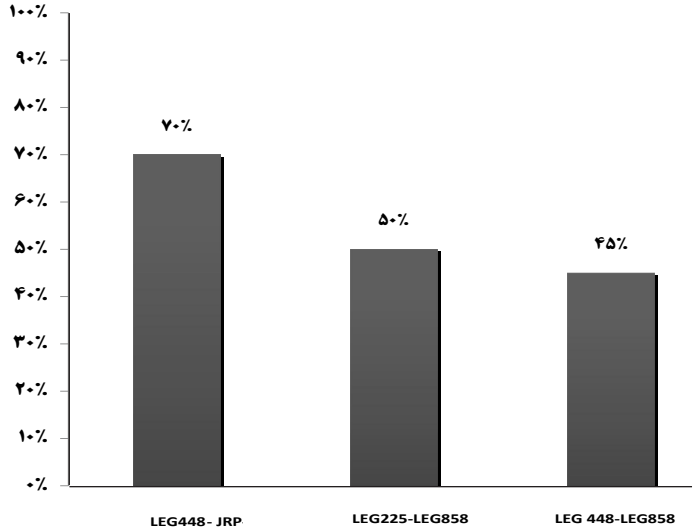
PCR p ƿʒ Ơǿƽĸƽ f ǰǿǘơ

ɛɬ ŀżƳĸÅǫƽɬ ĸ PCR ĸŽŽŮ Ơǿƽĸƽ f ǰǿš Ǫǵ ÃǿǂƐŢơ ǰǂɬ ʒƳ ĸ Ãŀĸãǫ ʒĸŧ ǰǂɬ ǻɬƐŀ.Ơəƨū ʒɬƐǎ ÆƽʒƐŀ ƳʒŃǫ ƳʒɬyŀĸǪƽɬ p Ńƽ DŽƱ ɬǫ ƠŏǫŮ ĸ ÉǂĸǻƨǪŧĸ ʒŃǿƽĦĸŚƽŃƽ yŀǂʒĸə Ŧǫ ƳʒɬyŀĸǪƽɬ ɛɬ Ĥ -Ĥǵ# cfu ƠŏǫŮ ɛɬ Æĺĸǻƨƽ ǻɛĸƽÃǿāʒ ĸŽŽŮ ĸ p yŀ ǴǿĦơ PCR ı ŀŧɬƿ p DNA ƨɬƨŲǪɬĸŽŽŮ 5 Śƽ .yǂƳƨū ǴǿĦơ ÊŀŃǂŤĸ .yŀ ǫĸ ŀĦ ƨŏŀ ƳʒŃǫ ÆǂĸǻƨǪŧĸ ǻĸǵƠāʒ ǻƿʒ ƨʎ

ǻʒĸǫd ʒŃǫƨd

ŀżƳĸÅǫƽɬ ƨǒĸĸŀd ɾǂĸǪǁ ƠĦΣ SPSS ǻʒĸǫd Ǵǫĸŀƨʎ ɛɬ ÃǿǂŢơ ǰǂɬ ʒƳ ŮĦŃŀĦ Ǵƽǂĸǂǫ ƠĦΣ McNemar ǻʒĸǫd ʒŃǫƨd ɛɬ .yǂƳƨū ɾǂĸǪǁ (P<ǵ/ǵĨ) ƨǂƳĸǂǫ ǻɬʎ .yŀ ŀżƳĸÅǫƽɬ ĸǵƨƨŽǂƨŦ ù ǪǁŲǫ .yŀ ǴǪəƨū ƨǒŀ ʒƳ ʒɬƳÆŀŀūǫ

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



ÊŀŃĠŤĸ ŮƽʒƐŀ ƠĦΣ ù ǪǁŲǫ ŭĸǵƨŽǂĠɬƨŦř ɛɬ ŀżƳĸÅǫƽɬ ĸ Ơǁĸǫ ǻĸǵ ǴŀŃŽŀ ÆŀĦƿɬƨə yDžƵƳ :Ħ ĸĞǁ

ĸǵǴǪəĸǻ

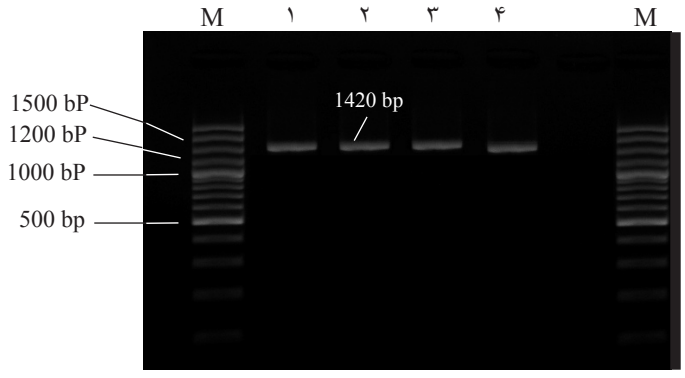
ƳʒŃǫ ÊŀŃǂŤĸ ƳŃΣƿ ÆƽʒƐŀ ƠĦΣ ǴŀŃŽŀ ĩǵ Ǵŭŀĸǻăǫ ǰǂɬ ʒƳ ÆƽʒƐŀ ɛɬ Ǩŀǁ yŀ ƨŧƵ Ǵŧ ʒŃǺ ŽĸŽǵ .Ơəƨū ʒɬƨǎ ı ǻĸǫƨd yŀĸǺ ƶǻĸƽ p ŮŃŀ ŀżyǵĸŦǫ ƠĦΣ ŮǴŭŀĸǻăǫ ƳʒŃǫ ÆǺd ǻĸǵǴŀŃŽŀ ǻĸǵƨƨŽǂƨŦř ĸ ǴǪəǻŀ ĸǵyǵĸŦǫ p ÆƽʒƐŀ ɛɬ yŭǺ Ǵŧ yǂƳƨū ŀżŀŲǫƽɬ ʒŃǒŀǫ ǰǂɬ ǫĸ ŀĦ ǻĸǵǴŀŃŽŀ ƳʒŃǫ ÆƽʒƐŀ p ÃǿǂŢơ ŮǴǪəǻŀ ĸǵǴǪəĸǻ PCR ΘèŃdžŢǫ ŀżyŀ ŀżyǵĸŦǫ ǻĸǵyŀĸǪƽɬ Ĥ ĸŮǁ .Ơəƨū ÊŀŃǂŤĸ ƳʒŃyŀĸǪƽɬ p Ńƽ ƠĦΣ ƨŽǂƨŦř ŮŃŀ Ǵƽ ƨǵ ɛɬ ŀżŀŲǫƽɬ ĸ ʒɬƨǎ ÆƽʒƐŀ ƳʒŃǫ ƨǿŀ PCR ĸŽŽŮ Ơǿƽĸƽ f ÃǿǂŢơ ǰǂɬ ʒƳ.Ơƽɬ Ơǿƽĸƽ f ǰǿǘơ ƠĦΣ ŮƨŽǂƨŦř ɾǂĸǪǁ ĥ ĸŮǁ ʒƳ Ǵŧ Ơəƨū Ǵŧ LEG448 -JRP ǻĸǵƨƨŽǂƨŦř ɛɬ ŀżŀŲǫƽɬ ĸ PCR p ƿʒ

ÙYfY ǯK‡ Ǻ ÆăØ[ǫ ǻKǵĠǺÑǯǺ ǯY ƗǯǺ ÆǺǻƵǯ OØ5Kƨ f ǰǻÇǫ‡Ø
.Oƨ‡ ǻy‖ Ġǻ‡ǯ‡
ǻKǵÇǯǻ‡Çř ç‡ǻYKǺǫ5‡KǺ OǺǺRǫ ǻKǵĠǺÑǯǺ ÆǺǺpǺ‡Çǻ yDžǯY ÙǺ ǨǨY
p (LEG225-LEG858) ‹(LEG448–JRP) ù ǫǫUǫ
.Oƨ‡ ǻy‖ Ġǻ‡ǯ‡ ÊǺÑǻŤǺ ÆƨǯǺ OǺΣ(LEG448-LEG858)
OǺΣ KǵĠǺÑǯǺ PCR ΘèÑdžŢǫ ƩǯÑǻpÇǫÙĹ‡ ħ ŘĞǁ ǯY
ƩÑǻpÇǫÙĹ‡ ƨǻǺǺǫ Ï ŘÙǁ ǯY p Kǵǻ Çǫ‡Kǻ ÆǫÑǯŰ DŽǺU ǂơ
ǻKǵÇǯǻ‡Çř ç‡ ǻYKǺǫ5‡ Kǻ ÊǺÑǻŤǺ ǻÇǫgKǻ OǺǺRǫ ǻKǵĠǺÑǯǺ
.Oƨ‡ ǻy‖ Ġǻ‡ǯ‡ JRP p LEG448

PŢǺ

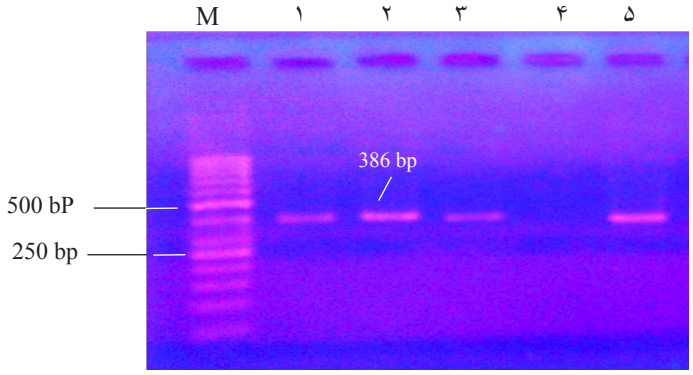
ÊØǻÑǫÑǺř ÊǺÑǻŤǺ ç‡ Æ‖KǺ ǻKǵǻǵǯKǯØǻ p ΘÊŨǂǫ ĠǺ ĠΣÑơKǻ
ǯ‡ÑǺŰ ĠǺ ÊǺÑǻŤǺ ĠǺ KǵǯKǫƨǯKǯØǻ kƋ ÆūYÑǺd Ġ‡ ǰǻ‡ ĠǺ ƐǒǺ p
Ġǫǻǯ ǯKǯ‖ ĠǺ ÆǺKǫƨǯKǯØǻ 5 ǻƐÑǫǺÑǻŤǺ DŽUǂǫ ŰǺÑǺ‡ ç‡ ÆŨǻ
ǻKǵ p pǯ ǰǻ‡ÇǺKƋǺ ÛOƨ‡ ÆǺǺKǫƨǯKǯØǻ ǻKǵOǺǺÑǻŰ ǻƐǫƐ ǯY p
.(ĥǵ)Oƨǻǯpǯlj ŘǫǺÑŰ ǰǻ‡ ŭǻƐƨ p ÃǺǻY ÆǻKƨKƋ‖
.OƨÊǺÑǻŤǺ ǻÇǫ‡KǺ ÆǻKƨKƋ‖ ǯY ƨKƋǫ ǻKǵ p pǯ ç‡ ÆŨǻ PCR
ÆǻèKǺ OǺƨKƨf ç‡ ÇŰǻY ǻKǵ p pǯ ĠǺ OǺƨ‖ p pǯ ǰǻ‡
ǪǵÛ(ĥĤ) Oƨ‡ ÆǺKǺǫƨY ŘǺŘǻ ŭǻƐƨ ǯƋ ƨǻKǫ‖ p ǻYÑǺ ǯ‡YǯÑǾÇǺ
ǯY ÊǺÑǻŤǺ ǻÇǫ‡KǺ ÆǻKƨKƋ‖ ǻ‡Çǻ p pǯ ǰǻ‡ ç‡ ǻYKǺǫ5‡ ǰǺƨš
º(ĥĤ)Oƨ‡ ĠǻÇDžĠǺ ǯpÇǻǫÇǫŭǺƨp 2 KǺǻǫ ǯY ÆăØ[ǫ ǻKǵĠǺÑǯǺ
ǫǺ‡Ǻ njǻÇ‖ ǯY‡t ĠǺǺHǺ KǺ ǻy‖ Æüƨ ĠüǺKăǫ ǰǻ‡ ǯY ǰǻÇǺKƋǺ

ƨǻKǫ‖ OãY Ù ł ƨKƋǫ ǻKǵÇǯǻ‡Çř kKUǫ‖ ĠǫǯƩΣ ç‡ PCR ‖ Ƌ‡ǫp
ǯY ÇǯǻÇř ǰǻÇơ ł ƨKƋǫ p ǻYfY ǯ‡Çǻ ÆƨǯÇǺ YǯÑǫ ƗǯKǵ ‖ ǻKǫǯd
.YÑ‖ ǻYfY DŽǺU ǂơ ÊǺÑǻŤǺ ǻÇǫ‡KǺ ÆǻKƨKƋ‖ ! ÑdžƟ
KǺ ÆǺd ǻKǵĠǺÑǯǺ ǯY ÊǺÑǻŤǺ ÆǻKƨKƋ‖ OǺΣ ĠǺǺǻǯ ǰǻ‡ ǯY
ǻYKǺǫ5‡ YǯÑǫ ù ǫǫUǫ ÇǯǻÇř ŰÑǺ Ġƨ ÛPCR p pǯ ç‡ ǻYKǺǫ5‡
ç‡ ǻYKǺǫ5‡ ƟǯÑdž ǯY Ġ‡ YfY ǯK‡ ǺǫDžKǻ ç ƨKƋǺ. Oǝ Çū ǯ‡Çǻ
ƟǯÑdž ǯY p KǵĠǺÑǯǺ ěǺĨ ¡LEG448-LEG858 ǻKǵÇǯǻ‡Çř
ÆǺp ÛKǵĠǺÑǯǺ ěĨǵ¡LEG225-LEG858 ǻKǵÇǯǻ‡Çř ç‡ ǻYKǺǫ5‡
yDžǯY ÛLEG448– JRP ǻKǵÇǯǻ‡Çř ç‡ ǻYKǺǫ5‡ ƟǯÑdž ǯY
p ǯ‡ƩǺ ÊǺÑǻŤǺ ĠǺ ǻYÑǺd ÛYÑǺ ěĪǵYpyƒ Ġ‡ KǵĠǺÑǯǺ ç‡ ǻÇǫ‡KǺ
.y‖y‖
ǻǯKǫd ǯÑǫƩd 2 Kƨ‡ ÇǺ KǵÇǯǻ‡Çř ù ǫǫUǫ ŰÑǺ Ġƨǻ‡ÆǺ YǯÑǫ ǯY
ǻKǵÇǯǻ‡Çř ç‡ ǻYKǺǫ5‡ ƟǯÑdž ǯY ǻǯYÆǺūǫ ÙÊǫƟH ÛMcNemar
ǻKǵÇǯǻ‡Çř ǰǺŢǯǵ p LEG448– JRPp LEG225-LEG858
y‖ ǻyǵKǂǫ LEG448– JRPp LEG448- LEG858
ç‡ ǻYKǺǫ5‡ ƟǯÑdž ǯY ǻǯYÆǺūǫ ÙÊǫƟH ÆǺp Û(P<ǵ/ǵǵĤ)
LEG448-LEG858p LEG225-LEG858 ǻKǵÇǯǻ‡Çř
.(P=ǵ/ĥĨ) yǻYÇŰǺǻyǵKǂǫ
DŽUǂǫ ĠǺƐū qKlǺ ǻKǵ ǁ ǻǫǯd ƨǻKǫǺ ĠǺ ĠΣÑơ KǺ ǰǻ‡ÇǺKƋǺ
ǻKǵÇǯǻ‡Çř ÇŰǻY ĠǺ OǺƨ‖ LEG448– JRP ÇǯǻÇř Ġƨ yǻYÇū
(LEG448-LEG858p LEG225-LEG858) ǻYKǺǫ5‡ YǯÑǫ
ĠǺ ÆǺd ǻKǵĠǺÑǯǺ ǯY ÊǺÑǻŤǺ ǻÇǫ‡KǺ ÆǻKƨKƋ‖ ǯY ÇpÑǫ ǻÇǯǻ‡Çř
.YpƵÆǫ ƵKǯ‖



ÆÅǺǫ ğÇǺǂƨ(Ĩ ÙOǺǺRǫ ğÇǺǂƨ (ħ¡PCRΘèÑdžŢǫ (ĦpĥpĤ ¡ (100bp) ÆĺÑÛĺÑǫ ÇūKƨKƋ‖ (M

⁎Ĥ/Ĩ ƩǯKūd ğţ ǻpǯ Ƈ KǵǻÇǫ‡KǺ ÆǫÑǯŰ DŽǺU ǂơ OǺΣ KǵĠǺÑǯǺ PCR ΘèÑdžŢǫ ƩǯÑǻ pÇǫÙĹ‡ :h ŘÙ‖



Ơ₪ğ ğ₫₪t(Ĩ ÛÁÅ¤ğ ğ₫₪t (ħ¡PCRΘèÑdžŢğ (₭₱ ĥ p Ĥ ¡(50bp) ÁĿÑÛĿÑğ ₫ūĸs₭₪‖ (M

xĤ/ Ĩɛp℥Ƙūd ğţ ắp℥ ₫Ⴏ **JRP℘ LEG448**ắ₭ģ₫℥₫₫ř ₭ Ê₪Ñ₴ŤĿ Á₴℥₫Ⴏ Ơ₭Σ ₭ģĠ₪Ñ℥₪ PCR ΘèÑdžŢğ ₫℥Ña p₫₪ÛĿ₭ :ĨĶÛ‖

ŮÑ₪ Ǵs ₫ģ p y₪‖₭y₪ ắ₫℥₭Ⴏ Θp₭Åơ (sharp ness) ţ Ñ1jp p Θp₭Åơ ŭãǂp ℥Y p Ůy₪YÑ₭ Á₪s₭₪ğ ắ₭ģy₪₭Ⴏ ₫₴y‖ ₫₴ÿğ₭ǂğ yĸ₭Ⴏ Ůã¤YÑ₭ Ġ₪Ñ℥₪ Æ₴₭s₭₪‖ p DŽ⁄₪Uǂơ ℥Y ù₪₪Uğ ắ₭ģ₫℥₫₫ř Ǵ₭ ₫₪ğ Ġt Ơs₭ ₫₴YĶÅ₪s₭ Y℥Ñğ ắ₭ģ₫℥₫₫ř Ơ⁄₪s₭ǂ f Ǵ₭ NjÑ₭₫ğ .YY₫ūÁğ ₫₴YÑ₭ĭd Ġ₪Ñ℥₪ Æ₴₭s₭₪‖ ǫyŰ K₴ p Á₴₭s₭₪‖

Ơ₪Σ Ǵ₪ə℥ ℥₭t Ǵ₭ p p℥ Ġt YĦY ℥₭ǂ‖ ĵ ⁄₪š Q̃g Ǵüĺ₭ãğ ĵ ₴ǂ ₴₴₭₪₪ Nj₭₫ğ ₴₴₭₪₪ Ġt ắǂĠ₪Ñū Ǵ₭ ₫₴YÑ₭ Æ₪s₭₪ğ p p℥ DNA ƧǂɛU₪s₭ Ơ₪₪ğ ₭ģĠ₪Ñ℥₪ ğ₭₫₫ğ₫₫ř ₭ PCR Ǵ₭ .y‖ p ℥ǂzū

ắɛ⁄₪ū Ǵ ₪⁄₪₪

p p℥ k₭U₪₪ ₭ Ġt YĦY ℥₭ǂ‖ ɛljĸf Ǵüĺ₭ãğ ɛǂ ĎDžĸf ₴₴₭₪₪

ǂ s₭₪ğ ắ₭ģ₫℥₫₫ř k₭U₪₪ p DNA ƧǂɛU₪s₭ Ơ₪Σ ǂ s₭₪ğ ǂ℥ Ê₪Ñ₴ŤĿ Ǵ₭ Æãơ⁄Ţğ ắ₭ģĠ₪Ñ℥₪ ÁūYÑ₭ĭd ÁūYĸs Ǵ₭ ℥₫Ñ₀Áğ ĎℨŮ Ơ⁄ŰɛS p ÆūŤ₴p ÛƠ⁄s₭ǂ f .YĦY DŽ⁄₪Uǂơ Ơ⁄ŰɛS Ǵ₭ 2 ĸSǂ ɛ⁄₭ Ġt Ơs₭ PCR ắĸ₴₴ğ ɛǂ Ûs₴₴₭₪₪ Ǵ₭ ÆĸĸÓ₪s¥ ℥Y è₭₭ ɛǂ ắɛ⁄₪ūÑ₪Σ Ơ₪Σ ǂ℥ ǂ s₭₪ğ ÁĿɛ₪₪t ΘĸğǂyĐǂ ℥₫Ñ₀Áğ ℥đ ! ĸ₫ ắ₭gnj⁄₪Ţğ ℥Y ₫Ť₴p Ǵ₭ ắɛ⁄₪ğ₭₫ ĵ ₴ǂ ɛǂ ĎDžĸf ắ₭ģƠ₪ÑÅÛ .YÑ℥₪ ǂɛΣǂ Ǵ₪DžĸəÊ₭

ɛǂ ₫₴YĶÅ₪s₭ ΘZÑDž ZY ÊĿÑ₴ŤĿ Ǵ₭ ₫₴YÑ₭ĭd Ơ₪₪ğ ắ₭ģĠ₪Ñ℥₪ Yǂy₪₫ ɛ₪ǂ₫₭ Á₪₫₫ Ĝ₫₫ĸ ₭ģɛ℥₫₫ř ɛ₪Ůã₴Y Ǵ₭ Ơ₪₪s‖ LEG448-JRP ɛ℥₫₫ř ắɛ₪ǂğ₭ Á₴₭s₭₪‖ ℥Y ɛ℥₫₫ř ĵ ₴ǂ YĶ₴ɛ Ơ⁄₪s₭s f ũãǂp ℥Y Ġt ₫₴YÑ₭ ℥Y Ġt ắǂĠüĺ₭ãğ ℥Y .y₪t₫ğ ℥₭⁄₭ ǂZ Á₫₫ ắ₭ģĠ₪Ñ℥₪ ℥Y Ê₪Ñ₴ŤĿ ěĬī Ġt y₴Yɛū DŽU ǂğ ƶ⁄₭ Ǵ₪əɛū ǫ₭ ₪‖ Ŧ₴ŤǪ₭ ℥Y ĥġ₫Ĩ ğ₭s ắ₭ģ₫℥₫₫ř ɛǂ ₫₴YĶÅ₪s₭ ₭ p Nested PCR p p℥ ₭ ₭ģĠ₪Ñ℥₪ ₭ Ġt Áĺĸƒ ℥Y Ůy₪₪s₫ Ơ₪₪ğ Ê₪Ñ₴ŤĿ ɛŏ₪ ɛǂ LEG448-JRP ₭ģĠ₪Ñ℥₪ ěĬī ¡LEG225 - LEG858ắ₭ģ₫℥₫₫ř ɛǂ ₫₴YĶÅ₪s₭ .(ĥĦ)y₪YÑ₭ ₫₴y‖ p ℥ǂzū Ơ₪₪ğ

℥₭ğɛ ℥Y ⁄ℨ ù₪₪Uğ ắ₭ģ₫℥₫₫ř Ǵs₴₭Ãğ Ơ⁄Űğǂǂǂǂ ĵ ₴ǂ ắ₭ģĠ₪Ñ℥₪ ɛ₪ŰƵt ĵ ⁄ŰƵƱơ ɛǂ ₭₭₪₪Σǂ Ơ₪Σ Á₫₫ ắ₭ģĠ₪Ñ℥₪ Á₴℥₫Ⴏ Á₴℥₫Ⴏ Ơ₪Σ Ġt Á₫₭ūĺ₭ãğ ℥Y .yğYÁğ ℥ǂɛ̃ā y⁄₫t₭ơ Y℥Ñğ ÛƠ₪₪ğ ɛ℥₫₫ř ŮÑ₪ Ŧ₴ÛPCR p p℥ ɛǂ ắɛ⁄₪ū₫ɛĦ₫ ₭ ÆãơŤ₪ğ ắ₭ģĠ₪Ñ℥₪ Ơ⁄₪s₭s f ƠQŰ Ǵ₭ Y℥ĦY ğ₭Ž₪f ǂ YɛÓūÁğ ℥ǂɛā ₫₴YĶÅ₪s₭ Y℥Ñğ ℥ǂyÁğ ɛǂ ɛ₪Ž₫ Ů₫₴y‖ Á₴₭s₭₪‖ ₫₴YÑ₭ĭd p Ơ₪₪ğ ắ₭ģĠ₪Ñ℥₪ Ůĵ⁄₴₭ř ℥Y Q̃g Ůù₪₪Uğ ắ₭ģ₫℥₫₫ř ɛǂ ₫₴YĶÅ₪s₭ ĵ ₴ǂ₫₭₫₫ .y‖₭ Ášũãǂp Á₴₭s₭₪‖ Ǵ₭ ɛ℥₫ğ ÛÁ₫₫₭ ắ₭ģĠ₪Ñ℥₪ Q̃g p Ášã⁄Ţğ ắ₭ģĠ₪Ñ℥₪ ℥Y Ġt y‖ yğǂÑƵ Yĸ₴ɛ ắ₭g₴ūŤ₴p p Ơ⁄₪s₭s f ₭ Á₴₭gģ₫℥₫₫ř ắ₭ãƵ ℥₭š Y ₫y‖ Á₴₭s₭₪‖ ₫₴YÑ₭ĭd ắ₭ģĠ₪Ñ℥₪ Yǂy₪₫ ΘZÑDž ĵ ₴ǂ .(Ī)YÑ₭ yğǂÑU₪ ắYĸ₴ɛ

Á₫₭s Ũ₴ nj₴₫ɛ‖ Ġt ĵ ₴ǂ Ǵ ǴΣÑƵ ₭ Ǵ₪əɛū ǫ₭ ₪‖ Ǵüĺ₭ãğ ℥Y y₪₫ ÛYÑ₭ Q̃t₭f Ķf₫ɛ̃ ǫ₭Ž℥ ℥Y PCR ‖ ₪₫₫p ǫ₭ ₪‖ Ơ₪Σ ₭ ₫₴y‖ ₫₴yğ₭ǂğ ắ₭gy₪₭Ⴏ ÛPCR ΘèÑdžŢğ ɛZÑəƤɛ₪ÛĿ₭ ɛǂ ℥YÑ₭ DŽU ǂğ ĬĸŤĺ ɛǂ ɛ℥₫₫ř Ơ₫Σ ŮÑ₪ Ǵs ɛ℥ ɛǂ ₫₴YĶÅ₪s₭

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

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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

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