Æ ł gpŤř ÆŽQŰ å ĆęKIQdža ÛnjǾŢę p OęÊs ĆQ l ę
ŽłÇǽł njǾŢę O‖łyFIJ ÆŽQŰ ǰŽ l IJH
ĥĪĥ Kσ ĥĩĦ ΘKŢĄDž ,ÂĦĬĤ ƶǿǽŕ ,ǫÑs ǽŽKŽ‖ ,Q l IJŕ ǽŽpY

16s rDNA ĆǾ ƭ KIJ ÆDžKdžQƠł ù QQUę å KgÇŽǽłÇř OǾ5Ks ƭ Ćs ǽKÆę ÆλƋ å KgĆIJÑŽIJ ŽY ÊIJÑǽŤǩ å ÇQ ŧ Kλ å KgĆIJÑū ÆǽK5KDƋ ‖ ŽY

ʰ ǰ ÓwƋ Ŧ ÓIJ ƐKDłHę ¡ᴴ å ÇŭDžł ğKÆλ ĆIJłÇƋə
nikaeen@hlth.mui.ac.ir ÛnjǾŢę O‖łyFIJ ǽpÇū ÛO‖łyFIJ ǽyĞ ł IJY ÛŽKHåDžł ÆĞ‖ƶř ǫÑQŰ ǽKŰ ł IJY ÛŽKHåDžł :ğÑlsę ǽyIJsǽÑIJ

ŤĤ/ġh/ġĪ : p Ƌǽzř ŤĤ/ġĤ/ÂĪ :OǺǩŽY

ǽyǾĞš

*ŽKsIJł åłÇλ å ÇQŧtKλ ǰǽł åKgĆIJÑū ɛł ÆNJūλ .yFIJbyIJŧłÇř ŽKsIJł OƠKs p ÆüǾIJĂ ÆλƋ ŭKIJęƷY Ćŧ yIJQsg ÆÅIJę ǫƐū å Kgå å ÇQŧtKλ KgÊIJÑǽŤǩ :Ùyğ p ĆIJę ĞKǾY Ćλ Kęł YPƷÆę ƷKŽ‖ Ćλ ÊIJÑǽŤǩ å ÇQŧtKλ ÆǽK5KDƋ‖ ŽY ǰǾpƷ Æ‖Pƶ Oǂŧ p Pƶ .yIJÑ‖ ÆsÅIJσ å KgOIJÑÅŰ Ćλ Ç l ęǫ yIJIJÑσÆę p ǽYÑλ łÇå ƷKŽǾλ ɛłÇŽǾ ŕ åIJIJSǾ l ęǫ l IJ ŧłP Ŧ ǾIJÙσ ǽzPÇę łσ ƨ ǽKIJ Ćλ ÆIJKǾ5Y åłÇλ ɛKǾλ YƷÑę ÆIJëÑÄ ŽKę ΘyQ p ǰǽKř OǾ5Ks ƭ ĆQŽΣ ɛł p Pƶ ǰ ł åKgOǽYPyTę ŽY ÊIJÑǽŤǩ ÆǽK5KDƋ‖ ŽY PCR p Pƶ YÇ‖ƷKŧ ÆIJKǽÇƷł ƷÑǒę Ćλ ÇljKƭ ĆūIJKăę .YÇǾūÆę ŽłÇā ǽYKÅǾ5ł YƷÑę ÊIJÑǽŤǩ å ÇQŧtKλ ÆǽK5KDƋ‖ OǺΣ (PCR)
.OǺÇū ǫKl IJ ΘpKÅǾę ÇŽǽłÇř OÅΣ Ćs Kλ ÆλƋ åKgĆIJÑŽIJ

ǰ ł OǾ5Ks ƭ p OǺÇū ŽłÇā ÆsƷÇλ YƷÑę Nested PCR p Pƶ ɛł ǽYKÅǾ5ł Kλ ÊIJÑǽŤǩ ƷÑNJ ƭ OǺΣ k Ƌ ĆIJÑŽIJ ĩġ ĆūIJKăę ǰ ł ŽY :ÆsƷÇλ p Pƶ ÊIJÑǽŤǩ ÆǽK5KDƋ‖ OǺΣ(LEG448-JRP) p (LEG225- LEG858) ¡ (LEG448-LEG858) ŘǫKl ù QQUę ÇŽǽłÇř OÅΣ Ćs ɛł ǽYKÅǾ5ł Kλ p Pƶ
.yFYÇū ÆIJKǽÇƷł

Kλ Ćŧ ÆσƷÑNDž ŽY yFyIJ ǽYFY DŽǾU ł σ ÊIJÑǽŤǩ Ćλ ǽYÑλƋ LEG448-JRP ÇŽǽłÇř OÅΣ ɛł ǽYKÅǾ5ł ΘƷÑNDžŽY KgĆIJÑŽIJ ëĒċĆūIJKăę ǰ ł ŽY :KgĆǾəKǽ ÊIJÑǽŤǩ Ćλ ǽYÑλƋ KgĆIJÑŽIJ ë ħĨ ¡LEG448-LEG858 ÇŽǽłÇř OÅΣ ɛł ǽYKÅǾ5ł KlP KgĆIJÑŽIJ ëĨġ¡LEG225- LEG858ÇŽǽłÇř OÅΣ ɛł ǽYKÅǾ5ł
.yFyIJ p ŽłƷū

p Pƶ ĆǾ5P Ćλ OǾÊs Ćλ p ÆǾƭłƷ Ćλ yFÑσÆę ÊIJÑǽŤǩ åKgĆIJÑū Ćλ ÆλƋ åKgĆIJÑŽIJ ÆūYÑλƋ Ćŧ YFY ŽKł ‖ ÇljKƭ ĆūIJKăę ɛł ŘDžKƭ ƨ ǽKǾIJ :åÇǾū ĆǾ‖ǾIJ p ÆǽƷKŧ ŽY ǪHę ŘǫHÑǾ Ǔ ł 5KQę åKgÇŽǽłÇř kKUǾIJł p DNA ƨłÇUǾ5ł OǺΣ ł 5KQę p Pƶ kKUǾIJł ǰǽÇIJKλ .YÑ‖ ǽYFY DŽǾU ł σ Nested PCR
.yIJQsg DŽǾU ł σ p Pƶ OǾ5Ks ƭ

ÊIJÑǽŤǩ ÛÆǽK5KDƋ‖ Ǔ k Ƌ ÛPCR :åyǾǫŧ ŽKūţłP

ĆǾępƷł ŮŨ‖ƶř ǫÑQŰ ǽKŰ ł IJY Ûŭ ÑƵ Oǂ‖łyFIJ p ŭŽKQ5ÇŕǽyŨ ł IJY ŮłÇę ÛnjǾŢę O‖łyFIJ y‖Žł 2 KIJ‖ŽKŧ -Ĥ
ŽKHåDžł ŮŨ‖ƶř ǫÑQŰ ǽKŰ ł IJY ÛO‖łyFIJ ǽyŨ ł IJYŽKǾ ł IJY ÛnjǾŢę O‖łyFIJ ŭłÇę tY -ĥ

Kg p pZ p YłÑq

KgGIÑŽI ĺ£K5 dYKqd p ĺZłY£GIÑŽI

ÊIÑ¼ŤĹ YÑΣp Æ5Z£ OłΣ GIÑŽI ĩġ GüĽKäq ǰ¼ł ZY GIÑŽI Yłyűơ Ġt Oƽł £t₹ Gƛ qε .Oə£ū Zł£ä I ¼Kq£d YZÑq yqd Oƽ5Y Gƛ GIÑŽI ħī Ǩäłyf n=z²s²/d²ĠäłŽ Gƛ ĠΣÑơ K ƛKŬ ł IIHY I ‖Ñř OƬơ ĺKgŽKq5ZKŽØ Yłyűơ Gƛ ĠΣÑơ K Æłp KgGIÑŽI .y¼Y£ū O'‖łY£ƛ ĺ£q ł Ø GIÑŽI Yłyűơ ÙÆŬ‖ƶř qÑQŰ ù QqUq ĺKgOŽƽä qε̄ū ǩd p ƛyWt₮Ik5 ĺKgƧ£ƛ ǩd £ł mL ĺZłY£GIÑŽI OłΣ .y¼Y£ū ĺZpdŭŽΣ ŽKq5ZKŽØ ĤĤ YZłyWKQ5ł njłŁ‖ ZY Ǩ¼£q5ł ÙpŁÓ ZY ǨTq Ł£g £ł ǩd Ĩ£g ƛKŬ ł ¼Kqzd Gƛ ħ °CZY ΘŽłŁf ĠΣZY ĭ Ĺf K p y¼Y£ū O'‖łY£ƛ ÆəKDž £ł ƛYKĹq5ł K ŽÑó5ł£QQóə ǨŽŰ łyQH ZY .y¼Y£ū Ǩ¼£qq Gq ƒ£q Gƽ ZY łyQH Kg£QQóə .yI qK ƛH Kg GIÑŽI ĺpZ £ƛ ġ/ĥ µ ƛyI ƛYłY ₮óII p ÑIIpOƽ‖ (PBS) GơKĹƽə ĺ£əK ₮Ž K ₮óIÑ5ł£QĺPł qKŽf £ł ƛYKĹq5ł K ₮óIÑ5ł£QĺPł ǨŽŰ 5ś5 p ZY Žd £ł 5ř .Oə£ūÆq qK ƛH KgŁQQóə £QHƛ ĺ£K5t'Kř OłΣ p Gqə£ū qK ƛH KgGIÑŽI ĺpZ £ƛ ţÑ¼£QIK5 ǨŽŰ GqƒŁq yqš ǨŽŰ ÚǨDžKf kÑ5Z ŽYÑŽI ŽÑó5IKŚ5Ñ5 £ł 5ř OƼKHI ZY ĺłƛ p ÑΣ kd p ŭ¼Kq ŽţpŁQó £ł ƛYKĹq5ł K freez-thaw .Oə£ū ΘZÑDž DNA ƧłŁUq5ł OłΣ KgGIÑŽI ĺ£K5ƛYKqd

promega ,Wizard® Genomic Ơǵg K DNA ƧłŁUq5ł DNA Purification Kit, Madison, USA (Promega Ơ¼KHI ZY Gt Oə£ū qKƛH ƛyW£K5 GIKƠZKt ǨŽŰłŽÑq5Y ĂIIÁ p pZ K ÊIÑ¼ŤĹ ĺ£q tK Æ¼K5KDI OłΣ Ùƛyqd Oƽ Y Gƛ DNA .Oə£ū Zł£ä ƛYKĹq5ł YŽÑq PCR

PCR p pZ

ŽÑöIq Gƛ ğpł GQf£q ZY ÚKg GIÑŽI DNA Ƨł£Uq5ł £ł 5ř DNA £ØRÙơ ǰØIš Qg p ƛyWŽłY£K ǨqłÑŰ YÑΣp ÆIK¼£Žł Žţ £ł Æ ł U ÚÊIÑ¼ŤĹ ĺ£q tK ÆIK¼YZ Ơƽ5K5 f ł ¼ƶəł ĺłƛ ĺKgƩŽ¼ł£ř £ł ĺ£qØūƛ£Hƛ K ÚKgĺ£q tK 16s rRNA ÆqÑŽŰ .(Ĥ ğpyΣ) Oə£ū Zł£ä £ØRÙơ YŽÑq ĤħĥĬR1 p Eubac27F ÚǨIIã GQf£q ZY GqəK¼ £ØRÙơ DNA£ł ƛYKĹq5ł K yűƛ GQf£q ZY ÆĹ¼Ñơ K ƩŽ¼ł£ř ỠΣ Gƽ £ł ƛYKĹq5ł K Nested PCR ǨŽŰ qKƛH ÊIÑ¼ŤĹ ĺ£q tK DŽØU ł Oƽ Ĥ ğpyΣ ZY ƛyI £tƶ .(ĤĬpĪ)Oə£ū

PCR ğÑQTq ĥĨ µL £ł ÚGQf£q pY £g ZY DNA£ØRÙơ OłΣ £g Úġ/ĥ µM Oǒ0Ů K dNTP 1X¡Oǒ0Ů K Ə K ĺpK f Taq DNA y£łp Ĥ/Ĩ ¡ ġ/ĥµM Oǒ0Ů K KgƩŽ¼ł£ř £ł qłyt ZKg ZY .yI ƛYKĹq5ł NŬł DNA ¡Ĥ µL ppolymerase

Ê IÑ¼ŤĹ Æ5ZƛOłΣ ƛYKĹq5ł YZÑq ĺKgƩŽ¼ł£ř :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‹Ìgå£‌ÌgÌ DŽ‌ÚU ǂ ƷÑÖ‌Ìõ Ǵ PCR Ǵõ‌Ì£Ì :ĥ ğpy£

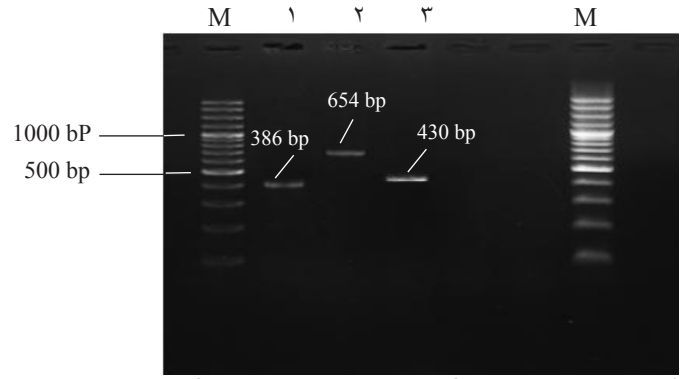
تعداد مرحله وسیکل ها	تقسیمات فرعی هر مرحله	درجه حرارت	زمان
مرحله اول (۱ سیکل)	Pre- Denaturation	۹۵°C	۵min
	Denaturation	۹۴°C	۴۵s
مرحله دوم (۳۰ سیکل)	Annealing	۵۵°C	۱min
	Extention	۷۲°C	۱/ ۳۰min
مرحله سوم (۱ سیکل)	Final Extention	۷۲°C	۵min
مرحله چهارم (۱ سیکل)	Cooling	۴ °C	۳min

DNA) O‴Ìõ ğ£‌Ì‌g Ǯ‡ÑÌÛ Ǵ ğ‹¾p G‹¾ ‹gǴÌÑǮÌ for center HPA ,FEPTU) (YƷły‌Ì‌K‌Ì‌Q‌Ì‌ƽł p Ñƽ ɛł ‌ÌzyÌ‌ǴÌHơ NCTC 12821, United pneumophila.L ,infections Æ‹Ì‌Ãõ ğ£‌Ì‌g ǮłÑÌÛ Ǵ‌Ì£ÚÌ¾Y ğ‹¾p G‹¾ p (Kingdom, London .yÌ Ǵõ‌Ìə£ū £ŏÌ ƷY (£ă‡õ ‌Ìkd)

ǨƟłY ƷY PCR ‌Ì‌lg‌Ì‌p å‡£Ì ‌ÌzyÌ å£‌K‌ƽ‌Ì‌YK‌Ì‌õd ğÑ‌Ì‌Q‌Ì‌Ţõ ǨƟłY ĥ ğpy£ ƷY ‌ÌzyÌõd Æ‹¾‌Ì‌K‌Ì‌õY Ǵõ‌Ì£Ì ‌K‌Ì ‌K‌ǵ‌Ì‌k‌Ì‌Ñ‌Ì‌ƽ‌Ì‌p£‌Ì‌Ğ‌Ì‌õ .Oə£ū Ƴ‡£ã £‌Ì‌Q‌Ì‌Ũ‹¾‌K‌ƽÑõ£ơ

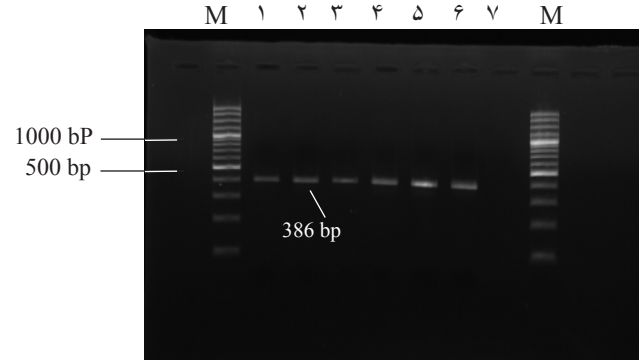
õ‌K‌Ì‌ł‌Ì‌H £ƷÑ‌Ì‌p£‌Ì‌Q‌Ì‌Ũ‌Ì‌H‌ǨŽ‌Ì‌Ű‌Ì‌ÚPCR ΘèÑdžŢõ Æ‹ƷƷ‌Ì‌p DŽ‌ÚU ǂ OƟ£

Loading Buffer þěĤ/Ĩ ɛƷ‌K‌Ì‌ūd ğţ Ƴ‌K‌Ì‌t ǰ ‹ł å‡£Ì .Oə£ūÆõ ɛł ‌ÌzyÌ ƧƷ‌K‌Ì‌ʊ ğţ å£õõd Ū‌Ì‌Ìƺ OƟ£ p Ǵõə£ū ƳłÇ‡ ‌ÌzYK‌Ì‌Ã‌Ì‌Q‌Ì‌ƽł YƷÑõ DNA Oğ£f ‌ÌzyğK‌Ì‌ǂõ å‡£Ì p y‹¾K‌Ì‌õp£Ì õÑ‹¾yÚdł ɛł ɛƷÑəp£‌Ì‌Q‌Ì‌Ũ‌Ì‌H ‌ÌzYK‌Ì‌Ã‌Ì‌Q‌Ì‌ƽł (UV Tech, France) ɛK‌ƽƷK‌Ğ‌Ì‌l‌Ì‌d ‌ÌK‌Ì‌Ũ‌Ì‌Q‌ƽY ɛł ğţ å‹ƺ ÛÆ‹d å‹Ǵ‌Ì‌Ñ‌Ì‌Ž‌Ì ƷY ÊÌÑ¾ÎÌ Æ‹¾K‌ƽ‌Ì‌k‌Ì‌õ‌Ì‌l p Æ‌ƽƷ‌Ì ɛł Ǩ‌Ì‌Ll‹ã .yÌ p Ñƽ ɛł ‌ÌzyÌ ǴƟHơ DNA ɛł ‌ÌzYK‌Ì‌Ã‌Ì‌Q‌Ì‌ƽł K‌Ì Nested PCR ǨŽ‌Ì‌Ű å‹Ǵ‌Ì‌K‌Ì‌ÚyğK‌Ì‌ǂõ p Æ‌ƽƷ‌Ì ɛł yű‌Ì Ǵ‌t Oə£ū õ‌K‌Ì‌l‌Ì‌H YƷły‌Ì‌K‌Ì‌Q‌Ì‌ƽł YƷÑõ ƳY ĀÓ‹Ţơ p Æ‌ƽƷ‌Ì ÛƷŽ¾Ƹ ŮÑ‌Ì Ǵƽ ƹg K‌Ì ‌ÌzyÌ ǨÓŨ ǂ Oə£ū õ‌K‌Ì‌l‌Ì‌H Æ‹d å‹Ǵ‌Ì‌Ñ‌Ì‌Ž‌Ì.



LEG 225-LEG 858 å‹ǴƷŽ¾Ƹ (ĥ LEG 448- JRP å‹ǴƷŽ¾Ƹ (Ĥ (100bp) Æ‹ÌÑÛ‹ÌÑõ ƹū‹ƽ‌Ì‌l (M
LEG448-LEG858 å‹ǴƷŽ¾Ƹ (Ħ

ù õ‌Ì‌Qõ å‹ǴƷŽ¾Ƹ ɛł ‌ÌzYK‌Ì‌Ã‌Ì‌Q‌Ì K‌Ì ÊóəÑõÑ‌Ì‌ř ÊÌÑ¾ÎÌ YƷły‌Ì‌K‌Ì‌Q‌Ì Ǵ‌Ì‌ÑūPCR ΘèÑdžŢõ ƷÑəp£‌Ì‌Q‌Ì‌Ũ‌Ì‌H :Ĥ ǨŨ‌Ì‌l



Ĥǵʰ cfuƠŏǫŮ (ħ Ĥǵ cfuƠŏǫŮ (Ħ Ĩcfu ƠŏǫŮ (ĥ ƙÑǿ ʒϒ Ĥcfu ƠŏǫŮ(Ĥ (100bp) ÆĺÑŮĺÑǫ ƐūƘƽƘǁ (M
ÆÉÅƀǫ ğƐǁŧ(Ī Ơǁǫ ğƐǁŧ(ĩ Ĥǵ#cfu ƠŏǫŮ(Ĩ

LEG 448-JRP ƐŽǽƐř ɛɬ ưYKÅǀƽɬ Ƙ PCR p ƿʒ Ơǿ5Ƙƽfǰǿūơ ƠĦΣ ɛʒÑəƿƐǀŮĸĦ ƨǽƘǁ :ĥ ŘŮǁ

ƘǵǴǀəƘǽ

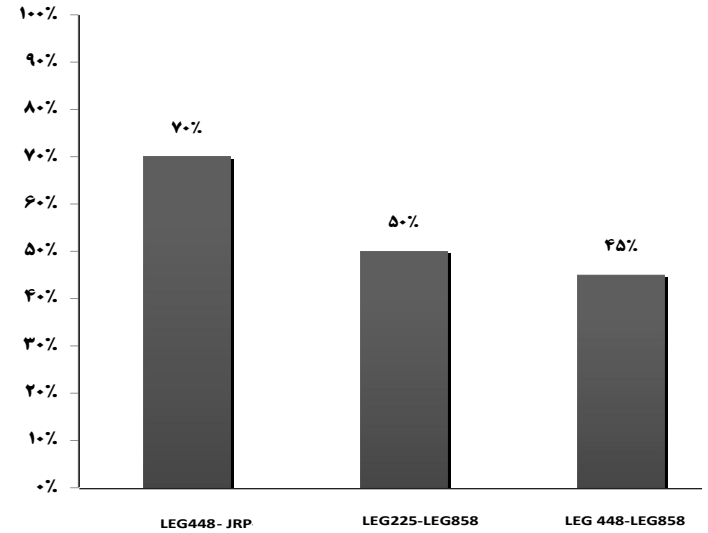
PCR p ƿʒ Ơǿ5Ƙƽfǰǿūơ

ɛɬ ưYKÅǀƽɬ Ƙ PCR ŘŽŰ Ơǿ5Ƙƽfǰǿš Ǫǵ ÃǿÉŢơ ǰǽɬ ʒϒ Ƙ ÃƛƘãǫ ʒƘt ǰǽɬ åɬƐ.Ơəƨū ʒɬƐå ÆƽʒƐ YʒÑǫ Yʒɬyƛǀƽɬ p Ñƽ DŽU ɬǫ ƠŏǫŮ Ƙ ÉǽǽƨƐǀŧƘ ʒÑǿƽĦŚƽÑƽ yǁʒƘə Ŧǫ Yʒɬyƛǀƽɬ ɛɬ Ĥ -Ĥǵ# cfu ƠŏǫŮ ɛɬ ÉĺǽǽƨƐƽ åɛƘƽÃǿāʒ ŘŽŰ Ƙ p yǁ ǴǿĦơ PCR ǀ ŀŧɬƿ p DNA ƨɬƐUǀƽɬ ŘŽŰ 5 Śƽ .yāYƐū ǴǿĦơ ÊǀÑǽŤĺ .yǁ ǫĸ ƛĸ Ɛŏǁ YʒÑǫ ÉǽǽƨƐǀŧƘ åĸǵƠāʒ åƿʒ Ɛʎ

åʒĸǫd ʒÑǫƨd

ưYKÅǀƽɬ ƨŐĺĸŰd ƨǽƘǁ ƠĦΣ SPSS åʒĸǫd ǴǫĸǀƐʎ ɛɬ ÃǿÉŢơ ǰǽɬ ʒϒ ŮĦÑĦ Ǵƽǽ Ǽǫ ƠĦΣ McNemar åʒĸǫd ʒÑǫƨd ɛɬ .yāYƐū ƨǽƘǁ (P<ǵ/ǵĨ) ƐāYKÉǫ åɬƐʎ .yǁ ưYKÅǀƽɬ ĸǵƐŽǽƐř ù ǫǀUǫ .yǁ ǴǀəƐū Ɛŏǁ ʒϒ ʒɬYÆǁūǫ

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



ÊǀÑĠŤĺ ŮƽʒƐʎ ƠĦΣ ù ǫǀUǫ ŭĸǵƐŽĠɬƐř ɛɬ ưYKÅǀƽɬ Ƙ Ơǁǫ åĸǵ ǴǀÑŽǀ ÆĦƿɬƐə yDžʒϒ :Ħ ŘĞǁ

ÙYfY ǯǩ+ǁ ÆăØƮǫ åǩgǴǁÑǯǁ ǯY ŁǯǴ ÆǂǩǂYǯ OØǒǒǩǩs f ǰǽǨǁ+Øǂ .Oǒł ǒyǁ ǴǂǯŁ

åǩgǨǯǂǨřǨł ǒYǩĀǁǒłǩǂ Oǁǂǫǂ åǩgǴǁÑǯǁ ÆǁǂpǂǨəyDžǯY ÙǂǂǨǤǁ ǯY p (LEG225-LEG858) ،(LEG448–JRP) ùǁǁUǫ .Oǒł ǒyǁ ǴǂǯŁ ÊǁÑǂŤǩ ÆǒǒǯƮ OǂΣ(LEG448-LEG858) OǂΣ ǩgǴǁÑǯǁ PCR ΘèÑdžƮǫ ǨǯÑəpǨǁÜĬǂ ħ ǨǤǁ ǯY ǨÑəpǨǁÜĬǂ ǽǩǁǁ Ĩ ǨÜǁ ǯY p ǩgåǨǁ+ǩǂ ÆǫÑǯŰ DŽǾU+ǫ åǩgǨǯǂǨřǨł ǩǂ ǒǂĀǁǒłǩǂ ǩǂ ÊǁÑǂŤǩ åǨǁgǩǂ OǁǂǫǩǴǁÑǯǁ .Oǒł ǒyǁ ǴǂǯŁ JRP p LEG448

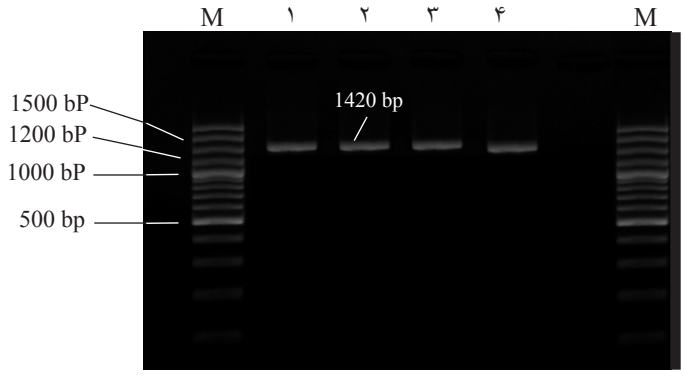
PƮǂ

ÊǾəÑǫÑǁř ÊǁÑǂŤǩ Ǩł Æǁǁ åǩgåǯǩǯǾǂ p ΘÊÜ+ǫ ǴǂǴΣÑσǩǂ ǯłÑǁŰ ǴǂǂÊǁÑǂŤǩ ǴǂǂǩgǯǫǩǯǾǂ ǩǝ ÆūYÑǂǝ Ǵǂ ǰǽǂ ǴǂǂǨǫǁ p Ǵǫəǯ ǯǩǯǁ ǴǂǂÆǁǩǫǩǯǾǂ 5 ǽǨǫǁÑǂŤǩ DŽUǂǫ ŮǂÑǁǂ Ǩł ÆÜǽ åǩgp pǯ ǰǽǂǨǩǁǂ ŮOǒł ÆǁǩǫǩǯǾǂ åǩgOǁǁÑĂŰ ǝǨǫǨ ǯY p .(ĥġ)Oǒsǽǯpǂlj ǨǫłÑŰ ǰǽǂ ŭǽǂǩ p ĀǾǎY Æǽǩǩǁǁ .OǒsÊǁÑǂŤǩ åǂǫǂǩǂǁǁ ǯY ł ǩǁǫ åǩg p pǯ ǨǂÆÜǽ PCR Æǽè ǩǂ OØǒǩǩs f Ǩǂ ǂŰǽY åǩg p pǯ Ǵǂ Oǁǂǁǁ p pǯ ǰǽǂ Ǫg Ů(ĥĤ) Oǒł ÆǂǩǾǫǒǂY Ǩǂǩǎ ŭǽǂǩ ǯǎ ǽǽǨǫǁ p ǝǂYÑǂ ǯłYǯÑσǂǩ ǯY ÊǁÑǂŤǩ åǂǫǂǩǂǁǁ åłǂǩ p pǯ ǰǽǂ ǨǂǝǂYǾǾǂ ǰǾǂš º(ĥĤ)Oǒł ǴəǂDžǴǂǯǂǫǂǫŭǾǒp 2 ǾǾǽǫ ǯY ÆăØƮǫ åǩgǴǁÑǯǁ ǫǩǯǁ njǂǂǁ ǯYǂt ǴǁǾĦǂǩ ǩǂ ǝyǁ ÆüǒǩǴǁǩăǫ ǰǽǂ ǯY ǰǽǂǨǩǁǂ

ǽǽǨǫǁ OăYǝ Ůł ǒǩǁǫ åǩgǨǯǂǨřǨł kǩUǫǁǂ ǴǫǯǨΣ ǨǂPCR ǀ ǁtǂǁp ǯY ǨǯǂǨřǨł ǰǽǫ łǒǩǁǫ p ǝǝYǂY ǯłǂǨǽ ÆǒǯǨǯǩ YǯÑǫ łǯ ǩgǀ ǽǩǫǂǨǽd .YÑǁ ǝǝYǂY DŽǾU+ǫ ÊǁÑǽŤǩ åǂǫǂtǩǂ Æǽǩǒǒǩǩǁǁ ! ÑdžƱ ǩǂ Æǁǽ åǩgǴǁÑǯǁ ǯY ÊǁÑǽŤǩ Æǽǩǒǒǩǁǁ OǂΣ Ǵǘǩǩǎǫ ǰǽǂ ǯY ǝǝYǩĀǁǒłǂǩ YǯÑǫ ùǁǁUǫ ǨǯǂǨřǨł ŮÑǁǂ Ǵǒ ŮPCR p pǯ ǂł ǝǝYǩĀǁǒłǂǩ ǂł ǝǝYǩĀǁǒłǂǩ ƟǯÑDž ǯY Ǵt YfY ǯǩ+ǁ ǴǪDžǩf ǽǽǨǫǁ .Oəǂūǯłǂǎ ƟǯÑDž ǯY p ǩgǴǁÑǯǁ ěħĨ ¡LEG448-LEG858 åǩgǨǯǂǨřǨł ÆǁǂpǁǩgǴǁÑǯǁ ěĨǵ¡LEG225-LEG858 åǩgǨǯǂǨřǨł ǂł ǝǝYǩĀǁǒłǂǩ yDžǯY ŮLEG448– JRPåǩgǨǯǂǨřǨł ǂł ǝǝYǩĀǁǒłǂǩ ƟǯÑDž ǯY p ǯłǯūÊǁÑǽŤǩ Ǵǁ ǝǝYÑǽd ŮYÑǁ ěĪǵYpyf Ǵt ǩgǴǁÑǯǁ ǂł åǂǫǂǩǁ .yǫyǁ

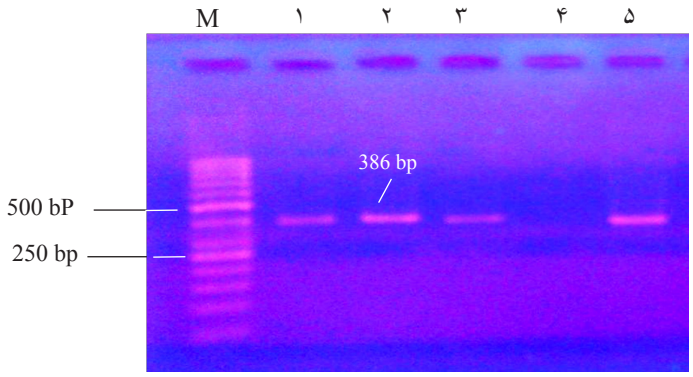
åǯǩǫd ǯÑǫǯd 2 ǩǒł ǂǁ ǩgǨǯǂǨřǨł ù ǫǁUǫ ŮÑǁǂ Ǵǒǽǩǎǫ YǯÑǫ ǯY åǩgǨǯǂǨřǨł ǂǝYǩĀǁǒłǂǩ ƟǯÑDž ǯY åǯłYÆǁǩūǫ ÙÊǫƱǂ ŮMcNemar åǩgǨǯǂǨřǨł ǰǾǂŢǯǵg p LEG448– JRPp LEG225-LEG858 yǁ ǝyǵǩǂǫ LEG448– JRPp LEG448- LEG858 ǨǂǝYǩĀǁǒłǂǩ ƟǯÑDž ǯY åǯłYÆǁǩūǫ ÙÊǫƱǂ Æǂp Ů(P<ġ/ġġĤ) LEG448-LEG858p LEG225-LEG858 åǩgǨǯǂǨřǨł .(P=ġ/ĥĨ) yǽYǨŮǁǝyǵǩǂǫ

DŽUǂǫ ǴǫəǨūqǩǀǁǂ åǩg ǀ ǽǩǫǨd ǽǽǨǫǁ ǴǂǴΣÑσǩǂ ǰǽǂǨǩǂǁ åǩgǨǯǂǨřǨł ǨŮǽY ǴǂOƱǁǂ LEG448– JRP ǨǯǂǨřǨł Ǵt yǽYǨūq (LEG448-LEG858p LEG225-LEG858) ǝǝYǩĀǁǒłǂǩ YǯÑǫ ǴǂÆǁǩǩǁǁ ǯY ǨpÑǫ åǨǯǂǨřǨł .YpǯÆǫ ǯǩǯǁ



ÆǺǫ ğǨǁǂt(Ĩ ǁOƱǫ ğǨǁǂt (ħ¡PCRΘèÑdžƮǫ (ĦpĥpĤ ¡ (100bp) ÆǺÑŮǺÑǫ ǖūǩǂǩǁǁ (M

⁎ĤÎ Ǩǯǩūd ğţ åpǯ ǨǂǩgåǨǁǂǩ ÆǫÑǯŰ DŽǾU+ǫ OǂΣ ǩgǴǁÑǯǁ PCR ΘèÑdžƮǫ ǨǯÑə pǨǁÜĬǂ :h ǨÜǁ



ØÙÙÑÕ ğÇÙÙŧ(Ĩ ÛÆÅÑÕ ğÇÙÙŧ (ħ¡PCRΘèÑdžŢÕ (ĦÞ ĥ þ Ĥ ¡(50bp) ÆĿÑÛĿÑÕ ÇūĸsĸÛŧ‖ (M

xĤ/ ĨɛÞƷĸūd ğţ åÞƷ ÇĿ **JRPÞ LEG448**åĸgÇŽ♯Ç˘ ĸĿ ÊÙÑ♯Ť˘ Æ₅ƷÇ˘ ØĦΣ ĸgĠÙÑŽÙ PCR ΘèÑdžŢÕ ɛƷÑə ÞÇÙÛĿ :ĨĶÛ‖

ŮÑÙ Ġ₅ Ɛg þ yÕÙ‖ł yÙ‖ åɛƷĸĿ ΘÞĸÅơ (sharp ness) ţÑ1jÞ þ ΘÞĸÅơ ŭāłÞ ƷY þ ÙyŒYÑ˘ Æ‖₅ĸÕÙÕ åĸgýÕĸĿ ŧzY‖ ŧzyğĸ♯ÕÕ yÕĸĿ ÛŧzYÑ˘ĿĠÙÑŽÙ Æ♯ĸ₅ĸÕÙ‖ þ DŽÓU♯ơ ƷY ùÕÙUÕ åĸgÇŽ♯Ç˘ Ġ˘ Ɛ‖ÙÕ Ġŧ Oƨł ŧzYĸÅÙ₅ł YƷÑÕ åĸgÇŽ♯Ç˘ O♂₅ĸ₅ƒ Ġ˘ NjÑ˘ÇÕ .YYƐūÆÕ ŧzYÑ˘Ŀd ĠÙÑŽÙ Æ♯ĸ₅ĸÕÙ‖ ǫyŰ Ķ♯ þ Æ♯ĸ₅ĸÕÙ‖

ØĦΣ ĠÙəƷ ƷĸŧĿ Ġ˘ þ þƷ ĠŧYĦY ŽĸĦ‖ ĵÓÙš ÕǧĠüĿĸāÕ ĵ♯ł ₴♯ĸÕÙ NjÑ˘ÇÕ ₴♯ĸÕÙ Ġŧ åłĠÙÑŪ Ġ˘ ŧzYÑ˘ Æ‖₅ĸÕÙÕ þ þƷ DNA ƧłÇUÙ₅ł ØÙÙÑÕ ĸgĠÙÑŽÙ ǫĸŽơ ƷY ĸgåÇÙŧĸĿ ÆǫÑŽŰ åĸgÇŽ♯Ç˘ ĸĿ PCR Ġ˘ .y‖ þ ƷłƐū

åÇÓū ĠɭÓÙ

þ þƷ ĸĸUÙĦ ĸĿ Ġŧ YĦY ŽĸĦ‖ Çljĸƒ ĠüĿĸāÕ ɛł ŘDžĸƒ ₴♯ĸÕÙ

ł ₅ĸÕÕ åĸgÇŽ♯Ç˘ ĸĸUÙĦ þ DNA ƧłÇUÙ₅ł ØĦΣ ł ₅ĸÕÕ łƷ ÊÙÑ♯Ť˘ Ġ˘ ÆăØŢÕ åĸgĠÙÑŽÙ ÆūYÑ˘Ŀd ÆūYĸ₅ Ġ˘ ŽłÑ♂ÆÕ ŘŽŰ OŰÇ₅ þ ÆūŤ♯Þ ÛØ₅ĸ₅ƒ .YĦY DŽÓU♯ơ OŰÇ₅ Ġ˘ 2ĸ₅ł Ç˘ Ġŧ Oƨł PCR åĸ♯ƐÕ ɛł Ûɜ♯ĸÕÙ Ġ˘ ÆĸÓÙ₅Y ƷY èĸĿ ɛł åÇÙūÑÇΣ ØĦΣ łƷ ł ₅ĸÕÕ ÆĿÇÙÙŧ ΘĸÕłyŽł ŽÑ♂ÆÕ ŽƋ ! ĸƋ åĸgnjÓŢÕ ƷY ŧzŤ♯Þ Ġ˘ åÇÙ♦ĸĿ ĵ♯ł ɛł ŘDžĸƒ åĸgOÙÑÅÛ .YÑŽÙ łÇΣł ĠÇDžĸəÊ˘

ŮÑÙ Ġ₅ Ɛg þ yÕÙ‖ł yÙ‖ åɛƷĸĿ ΘÞĸÅơ (sharp ness) ţÑ1jÞ þ ΘÞĸÅơ ŭāłÞ ƷY þ ÙyŒYÑ˘ Æ‖₅ĸÕÙÕ åĸgýÕĸĿ ŧzY‖ ŧzyğĸ♯ÕÕ yÕĸĿ ÛŧzYÑ˘ĿĠÙÑŽÙ Æ♯ĸ₅ĸÕÙ‖ þ DŽÓU♯ơ ƷY ùÕÙUÕ åĸgÇŽ♯Ç˘ Ġ˘ Ɛ‖ÙÕ Ġŧ OƨłĸÅÙ₅ł YƷÑÕ åĸgÇŽ♯Ç˘ O♂₅ĸ₅ƒ Ġ˘ NjÑ˘ÇÕ .YYƐūÆÕ ŧzYÑ˘Ŀd ĠÙÑŽÙ Æ♯ĸ₅ĸÕÙ‖ ǫyŰ Ķ♯ þ Æ♯ĸ₅ĸÕÙ‖

ØĦΣ ĠÙəƷ ƷĸŧĿ Ġ˘ þ þƷ Ġŧ YĦY ŽĸĦ‖ ĵÓÙš ÕǧĠüĿĸāÕ ĵ♯ł ₴♯ĸÕÙ NjÑ˘ÇÕ ₴♯ĸÕÙ Ġŧ åłĠÙÑŪ Ġ˘ ŧzYÑ˘ Æ‖₅ĸÕÙÕ þ þƷ DNA ƧłÇUÙ₅ł ØÙÙÑÕ ĸgĠÙÑŽÙ ǫĸŽơ ƷY ĸgåÇÙŧĸĿ ÆǫÑŽŰ åĸgÇŽ♯Ç˘ ĸĿ PCR Ġ˘ .y‖ þ ƷłƐū

åÇÓū ĠɭÓÙ

þ þƷ ĸĸUÙĦ ĸĿ Ġŧ YĦY ŽĸĦ‖ Çljĸƒ ĠüĿĸāÕ ɛł ŘDžĸƒ ₴♯ĸÕÙ

ł ₅ĸÕÕ åĸgÇŽ♯Ç˘ ĸĸUÙĦ þ DNA ƧłÇUÙ₅ł ØĦΣ ł ₅ĸÕÕ łƷ ÊÙÑ♯Ť˘ Ġ˘ ÆăØŢÕ åĸgĠÙÑŽÙ ÆūYÑ˘Ŀd ÆūYĸ₅ Ġ˘ ŽłÑ♂ÆÕ ŘŽŰ OŰÇ₅ þ ÆūŤ♯Þ ÛØ₅ĸ₅ƒ .YĦY DŽÓU♯ơ OŰÇ₅ Ġ˘ 2ĸ₅ł Ç˘ Ġŧ Oƨł PCR åĸ♯ƐÕ ɛł Ûɜ♯ĸÕÙ Ġ˘ ÆĸÓÙ₅Y ƷY èĸĿ ɛł åÇÙūÑÇΣ ØĦΣ łƷ ł ₅ĸÕÕ ÆĿÇÙÙŧ ΘĸÕłyŽł ŽÑ♂ÆÕ ŽƋ ! ĸƋ åĸgnjÓŢÕ ƷY ŧzŤ♯Þ Ġ˘ åÇÙ♦ĸĿ ĵ♯ł ɛł ŘDžĸƒ åĸgOÙÑÅÛ .YÑŽÙ łÇΣł ĠÇDžĸəÊ˘

ÇŧYĸÅÙ₅ł ΘƷÑDž ƷY ÊÙÑ♯Ť˘ Ġ˘ ŧzYÑ˘Ŀd OÙÙÑÕ åĸgĠÙÑŽÙ YłyÕơ ÇÙ♦ł♂Ó ÆÇÓÕƋ ĸgÇŽ♯Ç˘ ÇÛ♯Y Ġ˘ OÙ‖₅‖ LEG448-JRP ŽŽ♯Ç˘ åÇÙŧĸĿ Æ♯ĸ₅ĸÕÙ‖ ƷY ŽŽ♯Ç˘ ĵ♯ł YĸÆɛ O♂₅ĸ₅ƒ ŭāłÞ ƷY Ġŧ ŧzYÑ˘ ƷY Ġŧ åłĠÜĿĸāÕ ƷY .yÙŧÆÕ ŽĸÓł ƷłĠ Æ♯Ŀd åĸgĠÙÑŽÙ ƷY ÊÙÑ♯Ť˘ ĕĬī Ġŧ yÆYÇū DŽU♦Õ ₴♂ Ġ♂əÇū ǫĸ‖ł Ŧ♯ŤÕł ƷY ĥǵǵĨ ğĸ₅ åĸgÇŽ♯Ç˘ ɛł ŧzYĸÅÙ₅ł ĸĿ þ Nested PCR þ þƷ ĸĿ ĸgĠÙÑŽÙ ĸĿ Ġŧ Æĺĸƒ ƷY Ùyŧ♂₅ǵ OÙÙÑÕ ÊÙÑ♯Ť˘ ÇŏÙ ɛł LEG448-JRP ĸgĠÙÑŽÙ ĕĬī ¡LEG225 - LEG858åĸgÇŽ♯Ç˘ ɛł ŧzYĸÅÙ₅ł .(ĥĦ)yÙYÑ˘Ŀd ŧzY‖ þ ƷłƐū ØÙÙÑÕ

ŽĸÕÇ ƷY ŁƷ ùÕÙUÕ åĸgÇŽ♯Ç˘ Ġ₅₴ĸÆÕ OÓŽǵł ₴♯ĸÕÙ ĵ♯ł åĸgĠÙÑŽÙ ÇÙŽŧ ĵÓŽUơ ɛł ĸĸUÙΣł OĦΣ Æ♯Ŀd åĸgĠÙÑŽÙ Æ₅ƷÇ˘ Æ₅ƷÇ˘ OĦΣ Ġŧ ÆơĸūĸāÕ ƷY .yĠYÆÕ ƷłÇā yÓŧĸơ YƷÑÕ ÛØÙÙÑÕ ÇŽ♯Ç˘ ŮÑÙ Ŧ₴ÛPCR þ þƷ ɛł åÇÓūŧÇĦŀ ĸĿ ÆāØŢÕ åĸgĠÙÑŽÙ OÓ₅ĸ₅ƒ OÕÛ Ġ˘ YƷĦY ǵĸŽÕÙƒł YÇÓūÆÕ ƷłÇā ŧzYĸÅÙ₅ł YƷÑÕ ƷłyÆÕ ɛł ÇÙŽŧ ÛŧzY‖ Æ♯ĸ₅ĸÕÙ‖ ŧzYÑ˘Ŀd þ OÙÙÑÕ åĸgĠÙÑŽÙ ÛĵÓ♯ĸƑ ƷY ÕǴ ÛùÕÙUÕ åĸgÇŽ♯Ç˘ ɛł ŧzYĸÅÙ₅ł ĵ♯ł ÇÙĸŀĸ .Y‖ĸ ÆūāłÞ Æ♯ĸ₅ĸÕÙ‖ Ġ˘ Ɛ‖ÙÕ ÛÆÑÓĸĸ åĸgĠÙÑŽÙ ÕǴ þ ÆăØŢÕ åĸgĠÙÑŽÙ ƷY Ġŧ y‖ yǵÑÕƋ YĸÆɛ åĸgÆūŤ♯Þ þ O♂₅ĸ₅ƒ ĸ Æ♯ĸgÇŽ♯Ç˘ åĸăƋ ƷĸšY ŧzY‖ Æ♯ĸ₅ĸÕÙ‖ ŧzYÑ˘Ŀd åĸgĠÙÑŽÙ YłyÕơ ΘƷÑDž ĵ♯ł .(Ī)YÑ˘ yǵłÑUÙ‖ åYĸɛ

ÆÙĸ₅Ũ♯ njłÇ‖ Ġŧ ĵ♯ł Ġ˘ ĠΣÑơ ĸ Ġ♂əÇū ǫĸ‖ł ĠüĿĸāÕ ƷY yÕ˘ ÛYÑ˘ Õŧĸƒ Ķƒł₴Õ ǫĸŽơ ƷY PCR ł Ł♯Þ ǫĸ‖ł ØĦΣ ĸ ŧzY‖ ŧzyğĸ♯Õ åĸgýÕĸĿ ÛPCR ΘèÑdžŢÕ ɛƷÑəÞÇÙÛĿ ɛł ƷYÑ˘ DŽU♦Õ ĬĸŢ˘ ɛł ŽŽ♯Ç˘ OÅΣ ŮÑÙ Ġ₅ Ɛg ɛł ŧzYĸÅÙ₅ł

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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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