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DNA isolation by Chelex-100: an efficient approach to consider in leptospirosis early stages

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PEER REVIEW

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Comments

The article proposed a Chelex-100 assay as a rapid and effective approach for DNA extraction from clinical samples, and it can be used in leptospirosis diagnosis by PCR technology. The paper is in principle well written and the data are sufficiently presented.

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ABSTRACT

Objective: To compare the value of leptospiral DNA extraction procedures from clinical samples for the early diagnosis of leptospirosis.

Methods: Three DNA extraction procedures were applied for microbiological analysis, results of QIAmp DNA mini kit (QIAGEN, Germany), CLART HPV kit (GENOMICA, Spain) and Chelex-100 assay were compared concerning extraction efficiency, DNA purity and DNA suitability for amplification by specific polymerase chain reaction for pathogenic leptospires from blood, plasma and serum artificially infected.

Results: The comparison of extraction methods highlighted the efficiency of Chelex-100 and QIAmp DNA mini kit. Chelex-100 achieved the isolation of the highest concentration of leptospiral DNA from the culture and the spiked samples, with acceptable purities and without inhibitors to PCR.

Conclusions: Chelex-100 assay is a rapid and effective approach for DNA isolation in clinical samples having pathogenic leptospires and it could be useful in the early diagnosis of leptospirosis.

KEYWORDS

DNA extraction, Leptospirosis, Diagnosis, Chelex-100

1. Introduction

Leptospirosis is one of the main causes of acute febrile illness and is presumed to be the most widespread zoonotic disease in the world. Pathogenic *Leptospira* spp. can cause a widespread spectrum of disease in humans, which ranges from asymptomatic to a severe disease with rapid mortality. The clinical presentation in humans is difficult to distinguish from dengue, malaria, influenza, and many other diseases characterized by fever, headache, and myalgia[1].

Rapid diagnosis of leptospirosis, through culture and/or serology, can be difficult without proper expertise and is often delayed because of the time required to obtain results. Mainly those diagnosis techniques based on polymerase chain reaction (PCR) become molecular tools into valuable strategies due to their high sensitivity and specificity levels

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that often negate the need for isolation and culture of the infected *Leptospira* for a confirmatory result, and the results of such techniques are habitually available within a matter of hours instead of days or months[2].

Even though, successful PCR performance depends on a highly efficient DNA-recovery without PCR inhibitor remaining[3]; only little has been done to optimize these leptospiral procedures. In whole blood samples, a high recovery is especially important, since the density of bacteria is very limited. Further, only a small fraction equivalent to 5-10 μL of the original sample is included in the final PCR assay.

Traditional isolation procedures provide abundant quantities of highly purified DNA that generally exceed (both in quantity and quality) that required for successful PCR; however, they are relatively expensive, slow, and labor intensive, and they include multiple transfers and exposure to toxic chemicals[4]. Currently, commercial kits are fully used with encouraging results, but they are not available in all laboratories given their costs. To overcome these problems, alternative extraction methods incorporating improvements and simplifications of specimen process have been developed, remaining Chelex-100 one of the most used mainly in forensic application[5], although this chelating resin has been also used in formalin fixed and paraffin embedded tissues[6], blood[7] and swab samples[8] for microbiology testing.

The aim of our study was to determine the Chelex-100 effectiveness in recovering *Leptospira* DNA and in removing inhibitors in comparison with other DNA isolation methods, using spiked samples. The evaluation of Chelex-100 as efficient extraction system would therefore be of benefit in the improvement of strategies for early diagnosis of leptospirosis.

2. Materials and methods

Leptospira borgpetersenii serovar castellonis strain Castellon 3 was subcultured in Ellinghausen-McCullough-Johnson-Harris medium for seven days until reaching a cell density of 10^8 cell/mL approximately.

Blood was obtained from a healthy blood donor and infected with leptospiral strain in order to assure 10^5 cell/mL as final cell concentration. Five replicates of 1 mL serum, plasma and blood were achieved for each DNA extraction method to evaluate.

Both leptospiral culture and infected samples were subjected to DNA isolation procedures. Exponentially growing microorganisms were centrifuged at 14 000 r/min for 30 min at 4 °C, and the pellet was washed twice in phosphate-buffered saline. DNA was released from leptospires by either of the following methods:

Method 1: DNA was extracted using the QIAmp DNA mini kit (QIAGEN, Germany) according to the manufacturer's instructions. The final elution of DNA was done in 200 μL of elution buffer.

Method 2: DNA was extracted using the CLART HPV kit (GENOMICA, Spain), previously evaluated with success from leptospiral DNA in our laboratory (non-published results) and according to the manufacturer's instructions. The final elution of DNA was done in 200 μL of elution buffer.

Method 3: For serum and plasma, cell pellet was resuspended in 200 μL of 5% Chelex-100 in Tris ethylene diamine tetraacetic acid 1X solution and the extraction procedure was done as described by De Armas *et al*[6]. Otherwise, 200 μL of blood was mixed with 600 μL of 5% Chelex-100 for DNA extraction following the protocol mentioned intended for serum and plasma. Briefly, 5% Chelex-100 solution with clinical samples was vortexed and heated to 95 °C during 15 min, followed by a 12 000 r/min spin for 10 min. Finally, 5 μL of supernatant containing the DNA was used as template for PCR assays.

The quality of genomic DNA which recovered after each procedure was determined by analytic electrophoresis on a 0.8% agarose gel, which was stained with ethidium bromide (0.5 μg/mL) and visualized under ultraviolet light. DNA concentration was determined by spectrophotometry.

All DNA extracts from culture and spiked samples, were tested firstly by a PCR method detecting sample inhibition and this amplify a gene fragment encoding human β-globin (PCR β-globin)[9]; and then, the non-inhibited samples were subjected to the application of PCR method (PCR lipL32) targeting pathogenic leptospires[10].

The mean and standard deviation of concentration and purity values of DNA obtained by each extraction method were calculated. Comparison of means was performed using the statistical package EPIDAT 3.1.

This study was conducted in compliance with the Declaration of Helsinki and it was approved by the Institutional Ethical Committee. In addition, informed consent of blood donors was obtained and anonymity was preserved.

3. Results

DNA concentration achieved from leptospire cultures and infected samples are given in Table 1. Moreover, agarose gel electrophoresis demonstrated comparable amounts of the

appropriate genomic DNA with the extraction methods tested.

Table 1

Purity and DNA concentration values from leptospiral culture and clinical samples.

DNA extraction method	Parameter	Culture	Serum	Plasma	Blood
Chelex 100	Concentration (μg/μL) (mean±SD)	21.7±0.1	12.2±0.1	13.2±1.4	21.1±4.3
	Purity from protein (mean±SD)	1.7±0.2	1.0±0.3	1.0±0.1	1.5±0.2
	Purity from organic solvents (mean±SD)	1.1±0.0	0.2±0.0	0.3±0.1	0.8±0.1
CLART HPV kit (GENOMICA)	Concentration (μg/μL) (mean±SD)	12.9±0.3	9.0±0.2	10.2±0.8	10.9±1.5
	Purity from protein (mean±SD)	1.8±0.0	1.2±0.0	1.1±0.0	1.1±0.0
	Purity from organic solvents (mean±SD)	1.2±0.1	1.0±0.1	0.8±0.0	0.4±0.1
QIAmp DNA mini kit (QIAGEN)	Concentration (μg/μL) (mean±SD)	14.6±0.4	6.9±0.4	10.1±1.0	9.7±2.2
	Purity from protein (mean±SD)	1.9±0.0	1.3±0.1	1.2±0.0	1.5±0.1
	Purity from organic solvents (mean±SD)	1.4±0.1	0.8±0.1	0.8±0.0	0.9±0.0

SD: Standard deviation.

PCR inhibitors or insufficient DNA quantity were not identified by PCR β-globin in the clinical samples infected except for when blood with CLART HPV kit was used. Subsequently, the behavior of leptospiral DNA detection by PCR lipL32 was similar to founded one during inhibition assessment; hence, just blood with CLART HPV kit did not allow the visualization of expected electrophoretic band (Figure 1).

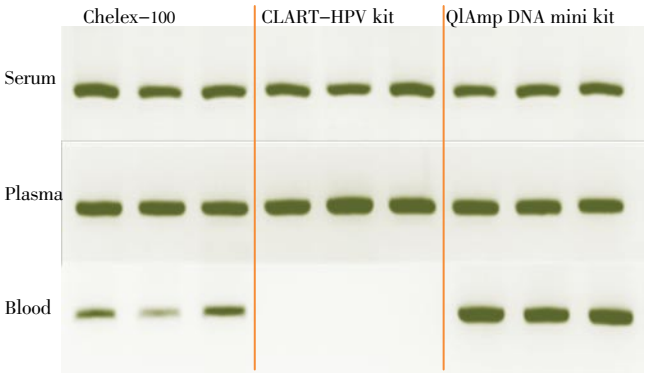

Figure 1. PCR lipL32 results in spiked samples using different DNA extraction methods.

4. Discussion

This study describes the usefulness of Chelex-100 as an efficient method allowing leptospiral DNA isolation in most frequent clinical samples used for leptospirosis diagnosis. Despite the simplicity attached to lacks of precipitation and purification steps of Chelex-100 procedure, values of DNA concentration and purity are acceptable compared with commercial kits, in fact the highest concentration values were obtained by Chelex-100, phenomenon was perfectly reasonable because DNA loss through columns in commercial kits may occur.

Chelex-100 is a chelating resin with a high affinity for multivalent metal ions. By binding to and removing ions during the isolation of DNA, damage to DNA (when heated) is prevented and inhibitors of Taq DNA polymerase are reduced. These two factors allow the successful use of minimally processed whole blood samples in PCR. DNA extraction procedure using Chelex-100 resin are very versatile, it does not require any organic solvent and the entire extraction procedure uses only two microcentrifuge tubes for each sample, decreasing the time for transferring of supernatants between tubes and reducing the possibilities of cross contamination[11].

After releasing DNA with any method, it is essential that any substances that may cause inhibition of the PCR amplification are successfully removed using a reliable, reproducible and sensitive purification procedure[11]. Consistent with this notion, PCR inhibitors or insufficient DNA quantity detected when blood with CLART HPV kit was used is explained probably by the reduced pore size of column present in CLART HPV kit, which is designed for genital samples not for blood containing red cell of large size, preventing DNA recovery.

The use of an extraction control has allowed the demonstration of the high performance of DNA isolation procedures, mainly Chelex-100, in the samples studied.

In addition, during the artificial infection procedure, 10^5 cell/mL was used consistently as described by Agampodi *et al*. where bacterial load in serum/blood ranged from 10^2 to 10^6 leptospires/mL in patients with leptospirosis in acute stage[12].

Reliable results found with lipL32 PCR reinforce the accurate application of Chelex-100 when blood, serum and plasma were used as matrix, and they also emphasize that this PCR method is not only applied for paraffin-embedded tissues as reported previously[10].

In summary, the practical advantages of sampling and storing blood for analysis of pathogen genes highlight the need for reliable, sensitive and cost-effective DNA extraction methods. The Chelex-100 DNA extraction method evaluated in this report has shown similar results compared with two commercial methods for DNA extraction. However, Chelex-100 procedure is rapid, simple, and inexpensive, and confers a reduced risk for cross-contamination due to minimum manipulation of samples during extraction. Future studies should be developed using clinical samples from leptospirosis confirmed patients in order to validate the DNA extraction method. The described method may therefore represent a useful tool in the early diagnosis of leptospirosis using PCR and other molecular studies.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

Leptospirosis is one of the main causes of acute febrile illness and is presumed to be the most widespread zoonotic disease in the world. Traditional DNA extraction method for diagnosis of leptospirosis are relatively expensive, slow, and labor intensive. The recommended DNA extraction method could be useful in the early molecular diagnosis of leptospirosis.

Research frontiers

The authors reported that there is a rapid, reproducible, and simple in-house method for the DNA extraction from blood, serum and plasma for diagnosis of leptospirosis and this method could be recommended for routine diagnostic PCR of leptospirosis in places with economic shortage, such as developing countries.

Related reports

Sample DNA extraction methods differ widely among laboratories. Unfortunately, very few papers addressing this issue have been published for leptospirosis and information about clinical specimens is insufficient.

Innovations and breakthroughs

The success of PCR depends on the quality of the extracted DNA. This paper propose the use of Chelex-100 as a rapid and effective approach for DNA isolation in clinical samples.

Applications

The recommended DNA extraction method could be useful in the early molecular diagnosis of leptospirosis.

Peer review

The article propose a Chelex-100 assay as a rapid and effective approach for DNA extraction from clinical samples, and it can be used in leptospirosis diagnosis by PCR technology. The paper is in principle well written and the data are sufficiently presented.

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