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# Rapid diagnosis of *Leishmania* spp. in blood samples from dogs using gold nanoparticles

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**Abstract**

There are many methods to diagnose Leishmania spp. a microscopic, serological or molecular method. However, finding a fast and accurate diagnostic assay is a necessary and urgent requirement nowadays. here, we improved nano detection method to diagnose Leishmania spp. In blood samples. In this study, we used a probe of oligonucleotide sequences associated with AuNPs 20 nm to diagnose different Leishmania spp. in blood samples of dogs. For this purpose, three series replicate of 10 µl of 23 ng / µl of Leishmania spp. DNA are used. A change in the solution colour to red is an indicator of a positive result, while the change to purple is a negative result. Then we compared these results with the molecular assay of IST1 gene. The overall relative sensitivity of the result is 90%. The result shows that 10 out of 10 samples of visceral Leishmania are positive with relative sensitivity and specificity 100%. Eight out of 10 samples of cutaneous leishmania are positive with relative sensitivity and specificity 80%. The present method is a fast and accurate method for detecting small amounts of DNA, and it is easy to distinguish by visual inspection.

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## Introduction

Leishmaniasis is a Zoonotic parasitic disease which is caused by protozoa parasites infect blood and tissues in humans and animals (1). There are about 20 human pathogenic species of *Leishmania* (2). It has two forms, the first one is mobile (Promastigote form), lives in the intestine of the female intermediate host sand fly (*Plebotomus* spp.), the another form is the amastigote form, which parasitizes macrophage cells in the skin, mucous membranes, lymph nodes, bone marrow, and spleen (3). Around 350 million people worldwide are infected with the Leishmaniasis, with an increase of 1.5-2 million cases annually (4). The primary diagnosis of the disease depends on its signs and symptoms. Likewise, the diagnosis of the parasite presence is confirmed by direct microscopic examination by direct smears of samples taken from the lesion or the culture medium, and the diagnosis goes away through the use of serological methods (5) or molecular methods (6). The unique high chemical and physical properties of gold nanoparticles make it the best in making probers capable of binding with sequences made of DNA is used in several fields, one of these areas is bio-sensory (7). The spectrum properties of gold nanoparticles and their ability to color change at a specified nanoscale range are used to detect sequences in a single or double strand of DNA (8). According to these characteristics, there are two methods of using gold nanoparticles, the first one is AuNP aggregation is induced by an inter-particle crosslinking mechanism which utilizes a three-component sandwich assay format, the second is based on the difference in the binding capacity between single and double DNA molecules (9). For the detection of *Leishmania*, the aggregation method of gold nanoparticles is used to detect the parasite in blood samples (10), as well as Andreadou *et al*. (11) are used the binding method to detect it in the culture.

The aim of the study is to find an easy and rapid method for detection *Leishmania* spp in blood samples of infected dogs and to compare the results with molecular methods.

## Materials and methods

### Sample collection

With help from veterinary doctor, 40 blood samples are taken from stray dogs with Leishmaniasis (20 with visceral and 20 with cutaneous Leishmaniasis). One ml of blood is drawn with a medical syringe from the edge of the ulcer (cutaneous Leishmaniasis) and from the vein (visceral Leishmaniasis), placed in EDTA tube and transferred to the laboratory. Presence of parasites was confirmed by ELISA test (12).

### Laboratory animals injecting

For this purpose, 25 Albino rats are used weight 250±2 g, are placed in special cages with adequate temperature and ventilation, and are fed with suitable feed. The rats are divided into three groups (13). The two experimental groups are included each group 10 rats, 5 rats representing the control group. The rats of the two experimental groups are injected with 0.5 ml of blood of infected persons (the first with the cutaneous and the second with the visceral) inside the peritoneal cavity and the footpad and without anesthesia, taking into account the injection of each animal with more than one sample. While the control group is left without injection, each group is marked with special numbers (14).

### Collect blood from laboratory animals

After 15 days of injection, all animals are anaesthetized by a sterile syringe 1 ml, with 0.2 ketamine and 0.1 xylazine. Three ml blood is drawn directly from the heart and placed in EDTA tubes, for DNA extraction.

### DNA extraction and primers

The primer and extraction kit is supplied from (Bioneer Company, Korea). Molecular extraction and examination are carried out according to the manufacturer's instructions. The extracted DNA is divided into two parts, molecular testing and biosensor nano-test. The primer for the ribosomal internal gene is selected transcribed spacer *ITS* (300-350 base pair) forward *LITSR* 5′ CTGGATCATTTTCCGATG 3′ and reverse *L5.8S* 5′ TGATACCACTTATCGCACTT 3′ (15,16).

### Nanoscale tests

Methods according to (Andreadou, et al., 2014) (11) is applied to detect *Leishmania* spp. by using the special prober of the (kinetoplast minicircle DNA) gene, which carries accession no. AF169140 in the gene bank (Table 1). The prober is manufactured and the melting and fusion temperatures are set in Al-Fadhel Company, Iraq.

Table 1: characteristic of selected probers

| Prober | Sequences | Sit of annealing | Melting Tm |
|---|---|---|---|
| LAu1 | GTTAGCCGATGGTGGTCTTG | 298-317 | 63.2 |
| LAu2 | ACGGGTGTCTTTGATGATGC | 251-262 | 63.8 |
| LAu3 | TAGTCTGGTGGGATGCTTCG | 223-242 | 63.2 |
| LAu4 | GTGCCTTTGATGTGGGTGTT | 148-167 | 63.5 |

### Preparation of gold nanoparticles and their hybridization with the prober

To prepare a solution at a concentration of 0.05 mg/ml, dissolving 5 mg of stock powder of gold nanoparticles in 100 ml of distilled water (17). According to method described by Andreadou *et al.* (11), AuNPs probes are constructed using 1 ml of AuNPs aqueous solution and 4 nmol thiolated oligonucleotides then binding with oligonucleotide probes Sequence. Therefore, three replicates of 10 µl of 23 ng/µl of *Leishmania* spp. DNA. All materials are prepared with (Nanoshell co. USA).

## Results

From 25 blood samples from which the DNA is extracted, the results of the molecular tests showed the presence of canine cutaneous and visceral *Leishmania* DNA in all animal samples of the two experimental groups, while the control group was negative (Figure 1).

The results of the nanoscale test shown that 18 of the twenty positive samples are positive by assay in the study method. Where the method is effective with all samples of visceral Leishmaniasis, while the result of two samples of cutaneous Leishmaniasis is negative, therefore overall relative sensitivity and specificity will be 90% (18/20), 100% and 80% of visceral and cutaneous Leishmaniasis samples, respectively. Statistically, the value of $x^2$ is 1.026 and the P. value is 0.311 between detection by the molecular method or the current study method (Figure 2).


Figure 1 Electrophoresis of 300bp PCR products of ITS1 gene. M Represents the volumetric index 2000-100 base pairs and samples 1-10 Positive for the parasite.

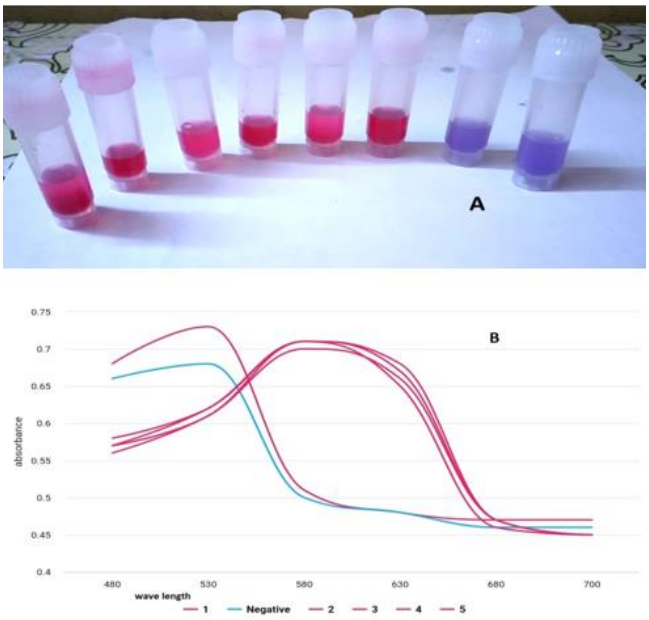


Figure 2 AuNPS assay result (red colour positive; blue colour negative).

## Discussion

The results of this current study showed the presence of *Leishmania* DNA in all samples examined using a primer *ITS1*. This results indicate a high specificity and relative sensitivity to this primer (18). The reason for such sensitivity and high specificity maybe because the researcher uses four probes, which gives less chance of a negative result (11). Also, the use of blood samples gives a greater chance for the presence of a lot of parasites, which affects the increase in sensitivity and specificity of detection (16).

Many of the physical and chemical properties that characterized radioactivity and Localized surface plasmon resonance (LSPR) have made it the best substance for used in the diagnosis and treatment of many diseases (19). The resonant activity of gold nanoparticles improves the radioactivity rate of the quantum emission. Thus, increasing the local color density or its variation towards a specific color (20). This color variation depends more on the quantity and quality of the target material, as the color density varies with different concentrations of the target material (21). Therefore, the goal of obtaining the highest testing accuracy is the ensuring of the nanoparticles reach the target sequence. For this, the researcher uses the thiol group as the lead group and the main component of AuNPs probe (10).

Several studies have indicated the possibility of using AuNPs in a different assay to detect the presence of *L. donovani* in blood or culture samples (22). Therefore, using blood samples to detect the presence of the cutaneous *Leishmania* by molecular methods or nanoparticles is the first attempt of its kind. Despite this, the results of this study are promising, as the specificity and high relative sensitivity of this experiment 90% is an indication for creating a fast and accurate method for detecting Leishmaniasis DNA (even in the case of cutaneous Leishmaniasis samples where the relative sensitivity of 80% is acceptable). Since this method depends on the target DNA molecules density, thus increasing the number of its molecules and improving its detection (22).

Based on the results of previous studies, the study uses 23 ng/µl of *Leishmania* spp. DNA in three series to confirm the effectiveness of this method in detecting the *Leishmania* spp. The concentration is the most effective because it contains DNA molecules suitable to start the chain of replication and doesn't produce high concentrations of DNA that may change the color of the indicator, thus affecting the result of the experiment (23).

## Conclusion

We can say that the method used is fast and accurate for detecting Leishmaniasis using a single DNA probe and associated gold nanoparticles. The easy of colorization of gold nanoparticles and the high ability to detect small amounts of DNA recommend this method as a successful and effective alternative method that can be used to detect many other organisms.

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## Conflict of interests

No conflict of interests is declared.

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# استخدام جزيئات الذهب النانوية في الكشف عن أنواع الليشمانيا في عينات دم الكلاب


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**الخلاصة**

هناك العديد من الطرق لتشخيص أنواع طفيلي اللشمانيا باستخدام المجهر او الطرق المصلية أو الجزيئية لكن، ايجاد طريقة تشخيص سريعة ودقيقة يعد مطلب ضروري. لذا كان الغرض من هذه الدراسة ايجاد مثل هذه الطريقة من خلال استخدام خاصية التغاير اللوني لجزيئات الذهب النانوية. تم في هذه الدراسة استخدام مجس من تتابعات مفردة من النيوكليوتيدات المقترنة مع الذهب ٢٠ نانومتر في تشخيص الأنواع المختلفة من اللشمانيا في الكلاب، واستخدم لهذا الغرض ثلاث مكررات من ١٠ مايكروليتر من ٢٣ نانوميتر/مايكروليتر من الحمض النووي المستخلص للشمانيا، يعد تغيير لون المحلول إلى اللون الأحمر دليل على النتيجة الإيجابية، أما تغيير اللون إلى اللون الأرجواني فيكون نتيجة سلبية. تم مقارنة هذه النتائج مع نتائج الفحص الجزيئي للجين *IST1*. كانت الحساسية النسبية الكلية لنتائج الاختبار ٩٠%، إذ أظهرت النتائج إن ١٠ من أصل ١٠ عينات لطفيلي اللشمانيا الاحشائية كانت ذات نتيجة إيجابية مع حساسية وخصوصية نسبية ١٠٠%، ثمانية من أصل ١٠ عينات للشمانيا الجلدية كانت موجبة الاختبار مع نتيجة حساسية وخصوصية ٨٠%. الطريقة الحالية طريقة سريعة ودقيقة في الكشف عن الكميات الصغيرة من الدنا، كما إنها سهلة التمييز بواسطة الفحص البصري.