Original Article

Assessing the Diagnostic Efficacy of a Developed Technique Utilizing Gold Nanoparticles in Diagnosis of Cystic Echinococcosis via the ELISA Method

Shirazi, S[1*], Zarrabi Ahrabi, S[2], Madani, R[3], Golchinfar, F[3]

1. *Department of Parasitology, Faculty of Veterinary Medicine, Science and Research Branch, Islamic Azad University, Tehran, Iran.*
2. *Department of Basic Health Science, Health Sciences Faculty, Marmara University, Istanbul, Turkey.*
3. *Department of Proteomics & Biochemistry section Biotechnology, Razi Vaccine and Serum Research Institute, Agricultural Research, Education and Extension Organization (AREEO), Karaj, Iran.*

How to cite this article: Shirazi S, Zarrabi Ahrabi S, Madani R, Golchinfar F. Assessing the Diagnostic Efficacy of a Developed Technique Utilizing Gold Nanoparticles in Diagnosis of Cystic Echinococcosis via the ELISA Method. Archives of Razi Institute. 2025;80(1):117-124. DOI: 10.32592/ARI.2025.80.1.117

ABSTRACT

Cystic echinococcosis (CE) is a significant zoonotic disease, transmitted primarily from canines to intermediate hosts such as humans, sheep, and cattle. This disease is caused by the larval stage of the Echinococcus granulosus tapeworm. CE can lead to severe health complications, including liver and lung cysts, which can cause organ dysfunction and even death if left untreated. The present study utilized serum specimens from 25 newborn babies as the negative control group, while an additional 25 serum samples were collected from surgically confirmed cases of CE to serve as the positive control group.The Iranian native antigen B was used to design the specific detection of hydatidosis in humans using an AuNP-ELISA method.The AgB isolated from sheep CE fluid was used for the design using the AgB-ELISA method. The study was meticulously executed in three stages.Initially, the commercial ELISA kit was employed. The second method entailed the utilization of native AgB to devise the ELISA method. Ultimately, gold nanoparticles (AuNPs) with anti-human conjugate combination and AgB-ELISA method were employed. The results demonstrate that the sensitivity and specificity for the diagnosis of hydatidosis were 92% and 100%, respectively, using the commercial ELISA kit; 96% and 100% with the AgB-ELISA method; and 100% with the Gold Nanoparticle-ELISA method.The utilisation of Ag B in ELISA design for hydatidosis diagnosis has recently garnered significant attention from researchers in the field. The findings suggest that incorporating AgB in ELISA design is highly advantageous; however, sensitivity can be significantly enhanced by utilising gold nanoparticles with anti-human conjugate, particularly in cases with lower titers that require high accuracy.

Keywords: ELISA, Nanoparticle, Antigen B, Diagnosis, Cystic Echinococcosis.

Article Info:
Received: 28 September 2024
Accepted: 15 January 2025
Published: 28 February 2025

Corresponding Author's E-Mail:
shahrokh.sh.63@gmail.com

1. Introduction

Cystic echinococcosis, a disease caused by the metacestode stage of *Echinococcosis granulosus*, is transmitted through the eggs of the parasite, which are shed in the faeces of infected canines. These eggs then infect intermediate hosts, such as humans and other domestic animals, including sheep and cattle, where the parasite develops into a hydatid cyst (1). According to the latest reports from the World Health Organization (WHO), cystic echinococcosis is endemic in South America, Eastern Europe, Russia, the Middle East, and China, with a relatively high incidence rate (2).In Iran, the prevalence of hydatidosis is reported to be 5% in humans (3), while in domestic animals, it varies between 3.1% and 16.4% across different regions (4). Given the significant public health implications of hydatidosis, prompt diagnosis is paramount. Conventional methods necessitate validation through additional assays exhibiting high specificity and sensitivity. Serological approaches have proven efficacious in this regard. The enzyme-linked immunosorbent assay (ELISA) has emerged as a particularly effective technique, offering the advantages of precision and expeditious results (5).AgB, a lipoprotein characterized by thermostability, gives rise to three distinct bands when subjected to immunological analysis. The subunits of AgB, when probed using immunoblotting, exhibit remarkable specificity and minimal cross-reactivity with other parasitic infections (6).In this study, an Iranian native antigen B was used to design the specific detection of hydatidosis using a specific ELISA method. Gold nanoparticles (Au-NPs) have high surface areas and unique physicochemical properties, which has led to their extensive use in the development of biomarker platforms (7). Their ability to combine with DNA, antibodies, enzymes and other biological molecules makes them effective amplifiers of biochemical detection signals. It is important to note that these nanoparticles can improve common biochemical diagnostic methods, and more interestingly, no special or expensive devices are required for this new method. Given the significance of nanoparticles in the diagnosis of various diseases, this new technology has not yet been widely adopted in the diagnosis of important parasitic diseases. Therefore, this study was conducted to investigate the use of gold nanoparticles in the diagnosis of hydatidosis.

2. Materials and Methods

2.1. Sample Collecting

A total of 50 serum samples were investigated in this study. Twenty five samples from newborn babies were used as negative controls, and another 25 samples obtained from pathologically confirmed cases of cystic echinococcosis (CE) following surgery were included as positive controls in the study. All serum samples were collected from the clinical laboratories of the East Azerbaijan province of Iran.

2.2. Extraction of Antigen B

A modified method was employed to extract antigen B from the hydatid cyst fluid. (8). The Bradford protein analysis method was used to measure the protein content of the prepared solutions. In addition, the solution containing the prepared antigen B was evaluated using SDS-PAGE.

2.3. Commercial Human ELISA

Indirect ELISA, utilizing various antigens, was employed to detect antibodies in the sera of CE patients. All samples were analyzed using a commercial human ELISA kit (prepared by Pishtaz Teb Company, Iran). A 100 µl volume of serum was added to the wells of a 96-well microplate at a 1:100 dilutions, and the plate was then incubated at room temperature for 30 minutes. Subsequent to this, the wells were washed and dried five times. In the next step, 100 µl of the anti-human conjugate was added to the wells and incubated for 30 minutes. Subsequent steps involved the addition of the Chromogen-Substrate and Stop Solution, respectively, and the monitoring of the reaction's absorbancy at 490 nm using an ELISA reader (Bio-Rad).

2.4. ELISA Method with Antigen B

The development of this methodology commenced with the identification of the optimal serum dilution level and the desired concentration of antigen B to be applied to the wells. This was achieved through the rigorous testing of varying antigen levels and serum concentrations. The preparation of serum dilutions involved the utilization of a potent positive serum and a negative serum. The initial dilution series was prepared at 1:50, 1:100, 1:200, and 1:300, with subsequent dilutions increasing to 1:250, 1:500, 1:1000, and 1:2000.The study utilized polyester wells (Nunc, Denmark). To achieve antigen binding, 100 µl of each antigenic concentration was added to each well, after which the plates were stored in the refrigerator overnight to allow the antigens to bind completely to the wells. The following protocol was employed to block the wells: after draining and washing three times by PBST, 250 µl of blocking buffer (skimmed milk, 5%) was added to the wells, which were then placed in a humid environment for 75 minutes in a 37°C incubator.In the subsequent steps, 100 µl of positive and negative sera were transferred into the wells with pre-prepared dilutions after three to four washes and drying. These were then incubated in a humid environment for 75 minutes at 37°C.The next step involved emptying the wells, followed by the preparation of 100 µl of anti-Human conjugate (manufactured by SIGMA USA at 1:30000 dilution) in the wells. This was carried out after five to six washes and drying steps, with the subsequent incubation occurring for 75 minutes in a humid environment at 37 °C.The final step involved a further five to six washes and drying of the wells. In this step, 100 µl of BM Blue POD Substrate (Roche Company, Germany) was added to the wells and placed in a dark environment for 12 minutes.Subsequently, 50 µl of the stop solution was added to each well.An ELISA reader equipped with a 450 nm filter was utilised to measure the optical absorbance of each well, and the absorbances of all wells were recorded. Due to the high sensitivity of the extracted antigen (AgB) in this study, a dilution of 0.5 µg/ml for the antigen and a serum dilution of 1:300 were used.

2.5. Nano-ELISA Method Using Antigen B

In order to devise the method, it is first necessary to ascertain the most suitable concentration of gold nanoparticle conjugate. The concentration of antigen B is fixed at 0.5 µg/ml, and antigen is bound to the wells. To prepare serum dilutions, a robust positive and a negative serum are used. In accordance with the hypothesis of this study, it is predicted that nanoparticles will increase the sensitivity of the test. In this stage, a serum dilution of 1:500 was used instead of a 1:300 dilution. In this method, all steps were performed similarly to the previous method (AgB-ELISA), except that the sera were used with a dilution of 1:500, and the conjugation with gold nanoparticles.

2.5.1. Preparing Gold Conjugate

In this study, the gold nanoparticle colloid (Plasma Chem, Germany) was utilized. To ascertain the most suitable dilution for the conjugate,five distinct concentrations of conjugate were prepared with four different dilutions (1:1, 1:2, 1:4, and 1:8). Five containers were labelled, and 1 ml of gold nanoparticles were transferred to them. In the subsequent stage, anti-human conjugates (SIGMA, USA) were amalgamated with these particles. To achieve this step, containers with gold nanoparticles were amalgamated with different amounts of anti-human conjugates on a shaker and inside ice for 30 minutes (Figure 1). Furthermore, 100 µl of the gold anti-human conjugate (Figure 2) at concentrations of 1, 1:2, 1:4, and 1:8 was transferred to wells and stored in a humid environment for 75 minutes in a 37 °C incubator. The subsequent steps were then conducted in accordance with the AgB-ELISA method (Figure 3). The optical absorption of each well was measured using an ELISA reader equipped with a 450 nm filter, and the absorbances of all wells were subsequently recorded.

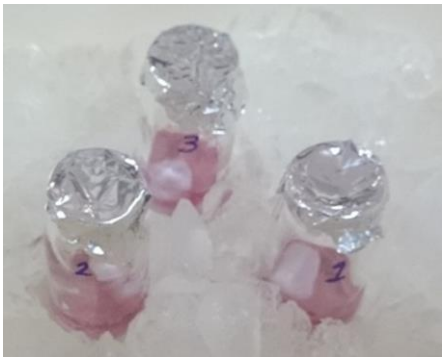

Figure 1: Some vials contain human conjugate and gold nanoparticles in ice (Original).

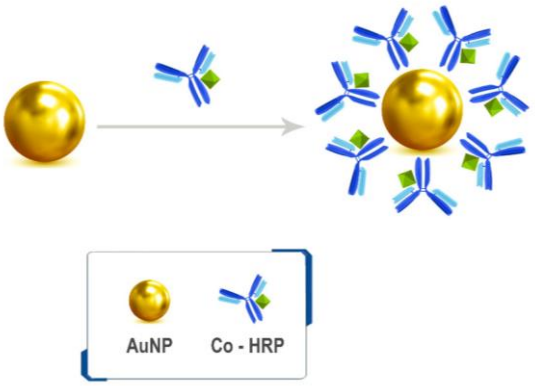

Figure 2. Combination of AuNP with human conjugates (Original).

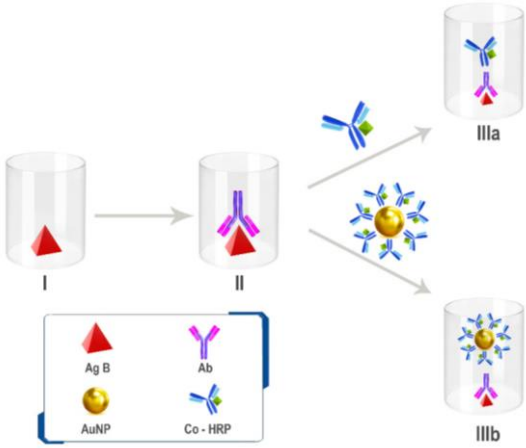

Figure 3. AgB-ELISA and AuNPs with AgB-ELISA (Original).

3. Results

The concentration of AgB was determined by Bradford assay, yielding a result of 0.7 mg/ml. The SDS-PAGE analysis revealed the presence of bands with a molecular weight of less than 15 KDa, which are indicative of AgB subunits (Figure 4).

3.1. Commercial ELISA Kit

The results obtained from the commercial ELISA kit demonstrate that all negative sera were found to be negative, and in the positive sera, two sera were determined to be negative (Table 1). According to the results presented in Table 2, the sensitivity and specificity of the commercial ELISA kit were determined to be 92% and 100%, respectively.

3.2. AgB-ELISA Method

Initially, a concentration of 0.5 µg/ml of AgB and a 1:300 human conjugate were utilised, with the employment of one positive and one negative serum sample to ascertain the optimal serum dilution.According to the findings presented in Table 3, it was determined that the most suitable dilution of serum was 1:300.Subsequently, the ELISA method was employed to examine definitive positive and negative sera, with the objective of identifying the cut-off value. The results obtained from this process indicated that the cut-off value was equivalent to one, signifying that sera with up to one positive result and below one optical absorption were designated as negative.Furthermore, the findings of this study revealed that the sensitivity and specificity of the ELISA designed with AgB for the detection of hydatidosis were 96% and 100%, respectively (Table 2).

3.3. Nano-ELISA Method

The AgB concentration was determined to be 0.5 µg/ml for binding to the end of the wells in the AgB-ELISA method. In order to minimize the interfering variables in this study, although it was not desired to change the amount of coated antigen, it was hypothesized that gold nanoparticles would increase the optical absorption and the detection quality. For this reason, serum dilution 1:500 was selected. The results presented in Table 4 indicate that the optimal dilution for the gold nanoparticle conjugate was conjugate No. 4, at a dilution of 1:8, which resulted in the most significant distinction in optical density (OD) between positive and negative sera. In the subsequent stage of the investigation, the cut-off level was determined through the application of a Nano-ELISA method. The results of this analysis indicated that the cut-off value was established at 0.8. Consequently, sera with an optical absorption reading of up to 0.8 were classified as positive, while those with readings below 0.8 were designated as negative. Consequently, all sera from sheep with positive hydatidiosis, and one of the sera from non-infected sheep, exhibited an optical absorption value greater than 0.8. The results obtained from the study indicated that the gold nanoparticles ELISA designed with AgB exhibited a 100% sensitivity and 96% specificity in detecting hydatidosis in sheep (Table 2 for details).

4. Discussion

Hydatid cyst is a prevalent zoonotic disease of international concern, recognized as a significant health concern in endemic regions, particularly in North Africa, South America, China, and the Middle East (9). In the last decade, the World Health Organization (WHO) has classified Echinococcosis granulosus as a distinct subgroup of selected Neglected Tropical Diseases (10).Epidemiological studies conducted across Iran have indicated an escalating trend of the disease (10, 11). Common serological methods for diagnosing hydatid cyst infection, ranked by sensitivity and specificity, include Enzyme-Linked Immunosorbent Assay (ELISA), indirect immunofluorescence, indirect hemagglutination, counter immunoelectrophoresis, and complement fixation test (Weinberg test) (12). In human medicine, serological methods are primarily utilized to corroborate radiological findings, with the ELISA method serving as an initial screening tool for serum analysis (13). Consequently, the antigens employed in the preparation of the ELISA kit must exhibit high specificity, with the WHO recommending one of these antigens, antigen B (8). Given the high sensitivity and specificity of the AgB-ELISA method and its increasing use in seroepidemiological investigations, it is important to use accurate methods for extracting this antigen, especially since this method is still primarily used in research facilities. Antigen B has been utilized in numerous hydatidosis seroepidemiological studies conducted in Iran (14). In 2013, researchers developed an ELISA kit employing antigen B and investigated the contamination with hydatidosis in various provinces of the country. Furthermore, hydatidosis has been confirmed with this antigen (AgB-ELISA) in several Iranian cities, including Shiraz (15). This method was employed to assess the seroprevalence of the infection in the region; however, no data is available regarding the sensitivity and specificity of the test. In contrast to humans, a few types of research have been conducted to improve the immunological methods for diagnosing hydatidosis in domestic animals such as sheep and cattle. The diagnosis of hydatidosis in natural hosts is basically done during autopsy (16). The accurate serologic diagnosis of infection in livestock is challenging due to cross-reactivity with other Cestoda strains, such as Taenia hydatigena and Taenia ovis.Furthermore, natural host animals exhibit a markedly weaker antibody response compared to the robust specific antibody production observed in humans. The use of diagnostic methods, such as CT scans and radiology, to detect the infection in intermediate hosts necessitates sophisticated equipment, which is not universally available. Conversely, serological methods, such as ELISA, are simple, beneficial, and cost-effective (13). Gold nanoparticles offer several key advantages, including their unique optical, physicochemical properties and high surface area, which facilitates dense antigen loading. AuNPs are biocompatible and can be readily conjugated with small biomolecules such as proteins, enzymes, DNA and amino acids (17).

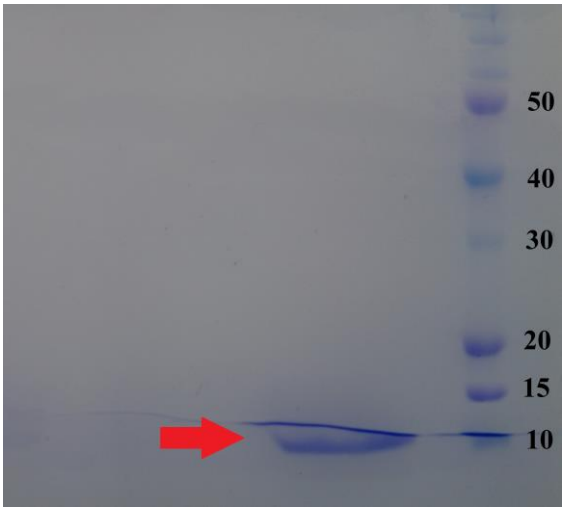

Figure 4. SDS-PAGE bands of Antigen B (indicated by the red arrow). The marker, showing the molecular weights in kilodaltons (kDa), is provided alongside the bands, and the molecular weight data were obtained (8).

Table 1. Optical density of Positive and Negative sera in commercial ELISA kit, AgB-ELISA and Nano-ELISA.

Positive Sera				**Negative Sera**			
No	**ELISA**	**AgB-ELISA**	**Nano-ELISA**	**No**	**ELISA**	**AgB-ELISA**	**Nano-ELISA**
Blank	0.085	0.065	0.085	Blank	0.101	0.101	0.176
C+	2.238	3.968	2.238	C+	2.377	2.377	3.271
C-	0.087	0.059	0.187	C-	0.112	0.112	0.235
1	2.317	3.361	2.148	1	0.133	0.31	0.331
2	1.178	1.888	1.154	2	0.125	0.479	0.435
3	1.641	2.446	1.934	3	0.279	0.326	0.286
4	1.164	2.107	1.831	4	0.32	0.387	0.299
5	1.21	2.348	1.981	5	0.101	0.305	0.405
6	1.479	2.284	1.906	6	0.165	0.367	0.467
7	1.242	2.467	1.811	7	0.099	0.396	0.399
8	0.384	1.099	1.228	8	0.12	0.339	0.454
9	1.051	2.096	1.989	9	0.096	0.354	0.498
10	2.951	1.991	1.211	10	0.116	0.373	0.233
11	2.052	2.095	1.895	11	0.159	0.327	0.359
12	1.155	1.155	1.139	12	0.213	0.298	0.298
13	1.805	2.171	1.987	13	0.099	0.212	0.282
14	2.192	2.481	1.961	14	0.102	0.335	0.371
15	1.254	2.354	2.339	15	0.122	0.296	0.384
16	2.165	2.231	2.411	16	0.088	0.386	0.599
17	0.391	0.469	0.912	17	0.145	0.577	0.377
18	0.973	1.052	2.073	18	0.096	0.398	0.398
19	1.987	1.687	1.993	19	0.11	0.213	0.218
20	2.134	1.117	1.883	20	0.114	0.315	0.276
21	1.649	1.347	1.829	21	0.289	0.378	0.394
22	2.279	1.211	1.058	22	0.178	0.491	0.381
23	1.391	2.354	1.953	23	0.121	0.303	0.261
24	1.289	1.286	1.434	24	0.135	0.335	0.415
25	1.433	1.895	1.345	25	0.202	0.202	0.257

Table 2. The comparison between commercial ELISA kit, AgB-ELISA and Nano-ELISA in Human sera.

		Commercial ELISA kit		AgB-ELISA method		Nano-ELISA method	
Case	**Serum**	**Positive**	**Negative**	**Positive**	**Negative**	**Positive**	**Negative**
Infected	25	23	0	24	0	25	0
Non infected	25	2	25	1	23	0	25
Total	50	25	25	25	25	25	25
Sensitivity		**92%**		**96%**		**100%**	
Specificity		**100%**		**100%**		**100%**	

Table 3. Serum Dilutions in AgB-ELISA methods.

Ag B	**0.5 µg/ml**		**1 µg/ml**		**2.5 µg/ml**		**5 µg/ml**		**10 µg/ml**	
Serum Dilution	Pos. Serum	Neg. Serum	Pos. Serum	Neg. Serum	Pos. Serum	Neg. Serum	Pos. Serum	Neg. Serum	Pos. Serum	**Neg. Serum**
1/50	----	----	2.931	0.541	3.391	0.449	3.366	0.398	3.379	**0.313**
1/100	----	----	3.180	0.441	*.***	0.381	*.***	0.306	3.265	**0.285**
1/200	----	----	3.413	0.367	2.739	0.318	3.109	0.253	3.182	**0.249**
1/400	----	----	3.247	0.305	*.***	0.255	3.277	0.250	3.277	**0.228**
1/250	*.***	0.551	4.445	0.515	----	----	----	----	----	**----**
1/500	3.197	0.445	2.994	0.521	----	----	----	----	----	**----**
1/1000	3.284	0.389	3.077	0.406	----	----	----	----	----	**----**
1/2000	2.734	0.445	2.970	0.347	----	----	----	----	----	**----**
1/300	**2.876**	**0.198**	----	----	----	----	----	----	----	----

Table 4. Dilutions for Gold nanoparticles conjugate.

No	**1**		**2**		**3**		**4**		**5**	
	Pos. Serum	**Neg. Serum**	**Pos. Serum**	**Neg. Serum**	**Pos. Serum**	**Neg. Serum**	**Pos. Serum**	**Neg. Serum**	**Pos. Serum**	**Neg. Serum**
Crude	2.745	0.832	3.124	1.103	3.123	1.154	3.789	1.002	3.321	1.003
1/2	2.129	0.612	2.451	0.596	2.703	0.879	3.310	0.701	3.001	1.234
1/4	1.746	0.451	1.989	0.435	2.012	0.568	2.981	0.671	2.987	0.820
1/8	0.814	0.320	0.879	0.376	1.412	0.381	**2.209**	**0.399**	3.110	0.509

A number of studies have explored the use of gold nanoparticles in the diagnosis of various diseases, including hydatidosis, and their antiparasitic specificity. However, to the best of our knowledge, there is no available data on the use of gold nanoparticles combined with antigen B for the diagnosis of hydatidosis. The unique biochemical properties of AuNPs, such as their optical versatility, tunable size and shape, convenient surface modification, biocompatibility, flexibility in fabricating different morphological forms, and ease of functionalization with active ligands via Au–S chemical bonds, have led to their emergence as the model system of choice for exploring a wide range of phenomena, including Biolabeling, drug carrier systems, catalysis, and DNA melting and assays (18, 19). Recent studies in the literature have demonstrated that nano-ELISA has been introduced as a highly sensitive protein detection method. Gold nanoparticles have been modified with a monoclonal detector antibody and HRP (signal molecules), with the gold nanoparticles acting as carriers and amplifiers (7). Recently, nanobiotechnology has been used to improve existing common methods for the diagnosis of various diseases (20, 21). However, the extant literature on this subject is limited to parasitology, with only a few studies available on the subject. In a study, gold nanoparticles and antigen B were used to design a Dot-Immuno-gold Staining (Dot-IGS) method, which showed that this method could be rapid and reliable. However, it cannot express sensitivity and specificity (22). In a subsequent study conducted in 2015, gold nanoparticles and EPC1 recombinant antigen were utilized to isolate anti-Echinococcosis granulosus IgG antibodies in dogs infected with E. granulosus. ELISA and Dot-Immuno-gold

Filtration Assay (DIGFA) were employed in this study, and the findings indicated that the ELISA method exhibited superior sensitivity and specificity (23). In this study, a comparison was conducted between the Commercial ELISA kit, the ELISA designed with antigen B, and the ELISA designed with antigen B combined with a gold nanoparticle conjugate. The results demonstrated that the Nano-ELISA method was more sensitive than the method without gold nanoparticles. The increased sensitivity of the Nano-ELISA method is attributed to the high surface-to-volume ratio of gold nanoparticles, which allows more antibodies to bind to the antigen-antibody complex. This enhancement leads to better pigmentation and improved detection capabilities. In conclusion, the employment of a robust and precise methodology can yield substantial benefits for seroepidemiological studies. The Nano-ELISA, conceptualized with native antigen B, has demonstrated remarkable efficacy in evaluating immune responses in intermediate hosts, such as humans, attributable to its exceptional sensitivity and specificity in epidemiological research. Furthermore, extensive research has employed antibody combinations to augment the functionality of AuNPs, a field that is rapidly expanding to offer a plethora of applications in the domain of medical science. Consequently, future research could further advance the diagnosis of various diseases using nanoparticles, including improved diagnostics for different parasites.

Acknowledgment
This work does not entail any specific acknowledgements.

Authors' Contribution
S.S study design and laboratory project. SZA contributed to writing and editing the manuscript. F.G collaboration in the laboratory and R.M study design and editing manuscript. All authors read and approved the final manuscript.

Ethics
It is hereby asserted that all samples were collected from laboratories, and that the process of sample collection did not constitute an interventional method. Furthermore, it is declared that all ethical standards have been observed in the preparation of the submitted article.

Conflict of Interest
The authors declare that they have no conflict of interest.

Funding
The authors confirm that they did not receive any financial assistance of this article's research, authorship, and/or publication.

Data Availability
The data that underpin the findings of this study are available upon request from the corresponding author.

References

1. Bhutani N, Kajal P. Hepatic echinococcosis: A review. Annals of medicine and surgery. 2018;36:99-105.
2. Brunetti E, Kern P, Vuitton DA. Expert consensus for the diagnosis and treatment of cystic and alveolar echinococcosis in humans. Acta tropica. 2010;114(1):1-16.
3. Mahmoudi S, Mamishi S, Banar M, Pourakbari B, Keshavarz H. Epidemiology of echinococcosis in Iran: a systematic review and meta-analysis. BMC Infectious Diseases. 2019;19:1-19.
4. Azami M, Anvarinejad M, Ezatpour B, Alirezaei M. Prevalence of hydatidosis in slaughtered animals in Iran. Turkiye Parazitol Derg. 2013;37(2):102-6.
5. Sadjjadi SM, Abidi H, Sarkari B, Izadpanah A, Kazemian S. Evaluation of enzyme linked immunosorbent assay, utilizing native antigen B for serodiagnosis of human hydatidosis. Iranian journal of immunology. 2007;4(3):167-72.
6. Haniloo A, Massoud J, Rokni M. Evaluation and comparison of antigen B-ELISA and antigen B-immunoblotting in immunodiagnosis of cystic hydatid disease. 2005.
7. Jia C-P, Zhong X-Q, Hua B, Liu M-Y, Jing F-X, Lou X-H, et al. Nano-ELISA for highly sensitive protein detection. Biosensors and Bioelectronics. 2009;24(9):2836-41.
8. Shirazi S, Madani R, Hoghooghi Rad N, Ranjbar Bahadori S. Isolation and purification of Echinococcus granulosus antigen B from hydatid cyst fluid using three different methods. Archives of Razi Institute. 2016;71(2):103-8.
9. Mandal S, Mandal MD. Human cystic echinococcosis: epidemiologic, zoonotic, clinical, diagnostic and therapeutic aspects. Asian Pacific journal of tropical medicine. 2012;5(4):253-60.
10. Fasihi Harandi M, Budke CM, Rostami S. The monetary burden of cystic echinococcosis in Iran. PLOS neglected tropical diseases. 2012;6(11):e1915.
11. Ahrabi SZ, Madani R, Bavili MM, Bedoustani AB. Incidence of cystic echinococcosis in the East Azerbaijan, Iran, during 2011-2017: a retrospective epidemiological study. Clinical and Experimental Health Sciences. 2021;11(1):158-62.
12. Barnes TS, Deplazes P, Gottstein B, Jenkins D, Mathis A, Siles-Lucas M, et al. Challenges for diagnosis and control of cystic hydatid disease. Acta tropica. 2012;123(1):1-7.
13. Rokni M. Echinococcosis/hydatidosis in Iran. Iranian J Parasitol: Vol. 2009;4(2):1-16.
14. Zarrabi Ahrabi S, Madani R, Shemshadi B, Ranjbar-Bahadori S, Hashemzadeh Farhang H, Montazer Bavili M. Diagnostic Value Of Antigen B In Hydatid Cyst By Dot-Blot Technique In The East Azerbaijan Province. Veterinary Research & Biological Products. 2019;32(2):34-41.
15. Asghari M, Mohebali M, Kia EB, Farahnak A, Aryaeipour M, Asadian S, et al. Seroepidemiology of human hydatidosis using AgB-ELISA test in Arak, central Iran. Iranian journal of public health. 2013;42(4):391.
16. Beard TC. The elimination of echinococcosis from Iceland. Bulletin of the World Health Organization. 1973;48(6):653.

17. Khan A, Rashid R, Murtaza G, Zahra A. Gold nanoparticles: synthesis and applications in drug delivery. Tropical journal of pharmaceutical research. 2014;13(7):1169-77.
18. Li W, Cao Z, Liu R, Liu L, Li H, Li X, et al. AuNPs as an important inorganic nanoparticle applied in drug carrier systems. Artificial cells, nanomedicine, and biotechnology. 2019;47(1):4222-33.
19. Sardar R, Funston AM, Mulvaney P, Murray RW. Gold nanoparticles: past, present, and future. Langmuir. 2009;25(24):13840-51.
20. Ambrosi A, Airo F, Merkoçi A. Enhanced gold nanoparticle based ELISA for a breast cancer biomarker. Analytical chemistry. 2010;82(3):1151-6.
21. Cho I-H, Irudayaraj J. In-situ immuno-gold nanoparticle network ELISA biosensors for pathogen detection. International journal of food microbiology. 2013;164(1):70-5.
22. Jahani Z, Meshgi B, Rajabi-Bzl M, Jalousian F, Hasheminasab S. Improved serodiagnosis of hydatid cyst disease using gold nanoparticle labeled antigen B in naturally infected sheep. Iranian journal of parasitology. 2014;9(2):218.
23. Kordafshari S, Hosseini SH, Jalousian F, Rajabibazl M, Jones M, Etebar F. Evaluation of dot immunogold filtration assay (DIGFA) by recombinant protein EPC1 for anti-Echinococcus granulosus IgG antibody. Iranian Journal of Parasitology. 2015;10(1):30.