African Journal of Biotechnology Vol. 9(31), pp. 5025-5027, 2 August, 2010
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB10.024
ISSN 1684–5315 ©2010 Academic Journals

Short Communication

Designing of enzyme linked immunosorbent assay (ELISA) kit for diagnosis copro-antigens of *Giardia Lamblia*

Afshin Barazesh[1]*, Jafar Majidi[2], Esmaeel Fallah[2], Rasul Jamali[2], Jalal Abdolalizade[3] and Reza Gholikhani[4]

[1]Department of Microbiology and Parasitology, Faculty of Medicine, The Persian Gulf Tropical and Infectious Diseases Research Center, Bushehr University of Medical Sciences, P. O. Box 3631, Bushehr, Iran.
[2]Department of Immunology and Parasitology, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran.
[3]Drug Applied Research Center, Tabriz University of Medical Sciences, Tabriz, Iran.
[4]Department of Biochemistry and Nutrition, Faculty of Medicine, Urmia University of Medical Sciences, Urmia, Iran.

Accepted 25 June, 2010

The sensitivity of microscopic examination of fecal samples to recognize *Giardia* parasites is low. In the methods based on antigen scanning of parasites such as enzyme linked immunosorbent assay (ELISA), copro-antigens of parasite will be traced and diagnosed even if the live parasite is absent in the fecal samples. To design this method, a pure antibody against parasite as well as an antibody conjugated to a proper enzyme is needed. In this study, an anti-*Giardia* IgG extracted from serum of contaminated rabbit was purified by ion-exchange chromatography and conjugated to the enzyme horse radish peroxidase (HRP). This antibody was used to design direct and indirect ELISA kits to measure conjugation titer. In both direct and indirect ELISA methods, optical densities (ODs) were 1 by using dilution of 1/4000 of conjugation. According to the results of both tests and the success in produced conjugate, it could be proceeded to prepare ELISA kits to diagnose giardiasis infections in various samples.

Key words: Enzyme linked immunosorbent assay (ELISA), antibody, copro-antigen, *Giardia lamblia.*

INTRODUCTION

Giardiasis is an intestinal infection with a worldwide spread caused by *Giardia lamblia* (Adam, 2001). Common method to diagnose giardiasis is microscopic examination of fecal samples (Rosoff and Stibbs, 1986). Disappearing parasite cyst in fecal samples will not always mean uprooting *Giardia* from the intestine of infected persons because this parasite has periodic expulsion in alternative days or various hours of day and also has short latent time in some patients and there is always a probability of hiding it by bile pigments (Gharavi et al., 2005). So the sensitivity of microscopic examination of fecal samples even with multiple examinations is low (50 to 70%) (Janoff et al., 1989; Burke, 1977). In patients who show clinical symptoms of giardiasis, ELISA is a rapid, sensitive, and economic method to confirm infection and copro-antigens of that parasite which could be traced and diagnosed even if the live parasite is absent in the fecal samples (Aldeen et al., 1995; Goldin et al., 1990).

For finding copro-antigens of parasite, anti-parasite antibodies could be used in a sandwich ELISA method. Comparing other diagnostic methods, sensitivity and specificity of this method is very high (about 100%) and could help in cases where the results of microscopic test are negative (Braga and Catapani, 2005; Duque et al., 2002; Fallahi et al., 2008). Nevertheless, the methods based on antibody search have ignorable uses in clinical diagnosis and the presence of antibodies especially in endemic places is not always a reason of infection because the results of these approaches would be positive even after months of

*Corresponding author. E-mail: afshin914@gmail.com. Tel: +98-917-186 0724. Fax: +98-771-2528724.

Abbreviations: HRP, Horse radish peroxidase; **TMB,** tetra methyl benzidine; **PBS,** phosphate-buffered saline; **ELISA,** enzyme linked immunosorbent assay.

Table 1. Conjugation titer levels using the designed direct ELISA kit. (parasite was coated to each well in constant quantity of 10^5/100 µl of PBS).

Conjugate Dilution	1/1000	1/2000	1/4000	1/5000	1/10000	1/20000
Optical Density	3.2	2.4	1.1	0.8	0.32	0.1

Table 2. Conjugation titer levels using the designed indirect ELISA kit.

Parasite quantity in 100 µl of PBS	Conjugate dilution						
	1/100	1/1000	1/2000	1/4000	1/8000	1/16000	1/32000
10^3	3	1	0.6	0.41	0.32	0.27	0.12
10^4	3.2	1.6	0.9	0.63	0.5	0.42	0.24
10^5	3.4	2.2	1.3	1	0.9	0.7	0.4

full recovery. In the method of parasite antigen finding, there is a need to pure antibody against parasite along with an antibody conjugated to a proper enzyme. So we had to conjugate the gained pure antibody against the parasite *G. lamblia* with a proper enzyme and by designing ELISA kits, we measured its acceptable titers by direct and indirect ELISA methods.

MATERIALS AND METHODS

In this fundamental-practical study, we purified parasite from faeces of patients and injected it into rabbit and extracted polyclonal antibody from serum of rabbit (Barazesh et al., 2007). Then the produced rabbit anti-*Giardia* IgG was purified by ion-exchange chromatography and its identity was confirmed by SDS-Page electrophoresis (Barazesh et al., 2007).

Conjugation

The purified antibody was conjugated by periodate method (Nakane and Kawaoi, 1974) that was slightly modified (Baradaran et al., 2006; Majidi et al., 2007). In this method, 5 mg of horse radish peroxidase (HRP) enzyme was mixed with 0.3 ml of 0.1 M sodium periodate and incubated at room temperature for 20 min. The mixture was then dialyzed by sodium acetate buffer (1 mM) at 4°C for a night and added to 0.5 ml of the mixture of 10 mg/ml of *G. lamblia* in sodium carbonate. After incubation for 2 h at room temperature, 100 µl sodium borohydride (4 mg/ml) was added to the mixture and incubated again at 4°C for 2 h. Finally, it was dialyzed several times by exchange against PBS overnight at 4°C.

Designing of direct ELISA kit

To measure the produced conjugation titer, both direct and indirect ELISA kits were designed. In direct ELISA method, 1×10^5 parasite cysts/100 µl to each well were used. Then they were incubated 45 min at 37°C and washed twice by PBS buffer and dried completely. The blocking procedure was done by adding 100 µl per well of 0.5% Tween and microplates were incubated at 37°C for 1 h. After washing, HRP-conjugated anti-*Giardia* IgG was added to the wells at dilutions of 1/1000, 1/2000, 1/4000, 1/5000, 1/100000, and 1/20000 and incubated at 37°C for 45 min and then were washed 5 times. 100 µl tetra methyl benzidine (TMB) was added to each well and incubated at room temperature for 20 minutes. Finally, block solution (sulfuric acid 5%) was added to wells and their optical densities (OD) were read at 450 nm by ELISA reader (State fax-USA).

Designing of indirect ELISA kit

100 µl of pure un-conjugated anti-*Giardia* antibody with dilution 1/100 was coated on each well and was incubated, washed and blocked using 10^3 - 10^5 parasite cysts/100 µl for wells. After incubation and washing, pure HRP-conjugated antibody in dilutions 1/1000 to 1/32000 was added to wells. The rest of the processes were like that in designing of direct ELISA kit; after incubation and washing, TMB was added and incubated. Block solution was added and their OD was read by ELISA reader.

RESULTS AND DISCUSSION

Direct and indirect ELISA kits were designed to measure the titer of produced conjugation and as shown in Table 1, OD of conjugation was above 1 for 10^5 parasites in 100 µl and 1/4000 dilution of conjugation (Table 1).

Table 2 shows that OD was above 1 for 10^3 parasites in 100 µl of PBS and 1/1000 dilution of conjugation as well as 10^5 parasites in 100 µl of PBS and 1/4000 dilution of conjugation (Table 2).

In the patients with giardiasis, *Giardia* has periodic expulsion in alternative days or various hours of day and its quantity will be decreased when the disease becomes chronic. Because of its short latent period, triggering clinical symptoms could be faster than beginning of cyst expulsion in some cases, and sometimes hiding the parasite by bile pigments could cause the parasite not to be seen in microscopic examination of fecal samples and subsequently result to false negative clinical report of the disease (Gharavi et al., 2005). Nowadays antigen scanning introduces better detection methods because it is believed that increase in antigen level of individual/sample shows that there is an increase in parasite level of infected individual/sample (Winiecka - Krusnell, 1995). In ELISA method, copro-antigens of parasite could

be traced and diagnosed even if the live parasite is absent in the fecal samples (Aldeen et al., 1995; Goldin et al., 1990). To design such method, along with a pure antibody against parasite, there is a need for an antibody to be conjugated to a proper enzyme.

To conjugate antibody to a proper enzyme, the usage instances should be considered. An enzyme that is proper to design ELISA kit could be unsuitable in some other objectives like immunochemistry. Furthermore, conditions of examination, pH, ionic strength, buffer composition and so on, could be incompatible with examination of sample and/or antigen-antibody reaction. In this study, the enzyme HRP was used to conjugate the pure antibody against *G. lamblia*. HRP is one of the most known enzymes in conjugation in serologic studies. Its molecular weight is about 44000 Da and there are various proper substrates for it in which their end product could be either soluble or insoluble. HRP is used in similar studies and the results of these approaches shows that a suitable enzyme is chosen (Majidi et al., 2007; Abdolalizade et al., 2008).

There are various methods to link an enzyme to an antibody (Nakane and Kawaoi, 1974; Yoshitake et al., 1982). To select the best method, it is important that the enzyme should be linked to antibody completely without deactivation of antibody and/or enzyme, the complex should be constant and whole for a long time and the method should be cheap and easy to use. HRP could conjugate to antibody by using each of periodate or CHM-NHS methods (Nakane and Kawaoi, 1974; Yoshitake et al., 1982). In this study, the antibody was purified by ion-exchange chromatography and then was conjugated by periodate method using previous (Baradaran et al., 2006; Majidi et al., 2007) approaches. In periodate method, the conjugation was done at carbohydrate end of each molecule and because its place has usually no effect on active site of enzyme and/or antibody, it is better than CHM-NHS method. Periodate also causes unfoldation of carbohydrate ring that could then conjugate to free amino groups (Wilson and Nakane, 1987). On the other hand, CHM-NHS is a method with two separate action that its maleimide links enzyme to thiolated antibody.

In this study, periodate was chosen as conjugation method and the results has shown that it was a correct choice. In direct ELISA test, in constant quantities of parasite and dilution of 1/4000, optical density of conjugation was above 1. In indirect ELISA test, OD was about 1 of dilution 1/4000 of conjugate and quantity of 10^5 parasite/100 µl of PBS. Both results show that the conjugation is correct and accurate. Referring to the gained conjugation, manufacturing ELISA kits to scan giardiasis infection in various samples should be worked on. This kind of test could be used as a proper screening test to detect asymptomatic carriers that expulse cyst and have main role in spreading of infection.

REFERENCES

Abdolalizade J, Majidi J, Farajnia S (2008). Production and purification of polyclonal antibody against human kappa light chain. J. Biol. Sci. 8(3): 683-686.

Adam RD (2001). Biology of *Giardia lamblia*. Rev. Clin. Microbiol. Rev., 14(3): 447- 75.

Aldeen WE Hale D, Robinson AJ, Carroll K (1995). Evaluation of a commercially available ELISA assay for detection of *Giardia lamblia* in fecal specimens. Diagn. Microbiol. Infect. Dis. 21(2): 77- 79.

Baradaran B, Majidi J, Hassan ZM, Abdolalizade J (2006). Large scale production and characterization of anti-human IgG monoclonal antibody in peritoneum of Balb/c mice. Am. J. Biochem. Biotechnol. 1(4): 190-193.

Barazesh A, Majidi J, Fallah E, Abdolalizadeh J, Jamali R, Ghazanchaei A (2007). Purification of Anti-*Giardia lamblia* by Ion-exchange chromatography. Int. Med. J. 6: 1

Barazesh A, Majidi J, Fallah E, Jamali R, Ghazanchaei A, Abdolalizade J (2007). Production of Polyclonal antibody against *Giardia lamblia* in rabbit. J. Ilam Univ. Med. Sci. 14(4): 25-30.

Braga Vidal AM, Catapani WR (2005). Enzyme-linked immunosorbent assay (ELISA) immunoassaying versus microscopy: advantages and drawbacks for diagnosing giardiasis. Sao Paulo Med. J. 123(6): 282-5.

Burke JA (1977). The clinical and laboratory diagnosis of giardiasis. Crit Rev. Clin. Lab. Sci. 7: 373-391.

Duque S, Santiago Nicholls R, Arevalo A, Guerrero R, Montenegro S, James MA (2002). Detection of *Giardia duodenalis* antigen in human fecal eluates by enzyme-linked immunosorbent assay using polyclonal antibodies. Mem. Inst. Oswaldo Cruz, Rio de Janeiro. 97(8): 1165-1168.

Fallahi Sh, Gharavi MJ, Qara-gozlou B, Sepahvand A, Mahouti F (2008). A comparative evaluation of Giardiasis prevalence by rutine parasitical assays and antigen detection in elementary school children in Delfan town, Iran. J. Lorestan Univ. Med. Sci. 4(9): 45-49.

Gharavi MJ, Fallahi Sh, Qara-gozlou B (2005). Evaluation of *Giardia* detection by rutine parasitical assays and antigen detection techniques. 5th National Iranian Congress of Parasitology. Tehran, Iran. p. 346.

Goldin AJ, Wener ART, Aguilera X, Zulantay I, Warhurst DC, Miles MA (1990). efficient diagnosis of giardiasis among nursery and primary school children in Santiago, Chile by capture ELISA for the detection of fecal *Giardia* antigens. Am. J. Trop. Med. Hyg. 42(6): 538- 545.

Janoff EN, Craft JC, Pickering LK, Novotny T, Blaser MJ, Knisley CV, Reller LB (1989). Diagnosis of *Giardia lamblia* infections by detection of parasite-specific antigens. J. Clin. Microbiol. 27(3): 431-435.

Majidi J, Abdolalizade J, Amirkhiz M B, Majidi S (2007). Production and purification of polyclonal antibody against bovine immunoglobulins in rabbits. Afr. J. Biotechnol. 6(12): 1369-1372.

Nakane PK, Kawaoi A (1974). peroxidase labeled antibody: A new method of conjugation. J. Histochem. Cytochem. 22(12): 1084-1091.

Rosoff JD, Stibbs HH (1986). Isolation and identification of *Giardia lamblia* specific stool antigen (GSA65) useful in coprodiagnosis of giardiasis. J. Clin. Microbiol. 23: 905- 910.

Wilson MB, Nakane PK (1987). In Immunofluorescence and Related Techniques, (Knapp W, Holubar H and Wick G. eds), *Elsevier,* Amsterdam.

Winiecka-Krusnell J (1995). Detection of *Giardia lamblia* cysts in stool samples by immunofluorescence using monoclonal antibody. Eur. J. Clin. Microbiol. Infect. Dis. 14(3): 218- 222.

Yoshitake S, Imagawa M, Ishikawa E, Urushizaki I, Nishiura M, Kanazawa R, Kurosaki H, Tachibana S, Nakazawa N, Ogawa H (1982). Mild and efficient conjugation of rabbit Fab and horseradish peroxidase using a maleimide compound and its use for enzyme immunoassay. J. Biochem. 92: 1413-24.