J Appl Biotechnol Rep. 2022 March;9(1):557-562

doi 10.30491/JABR.2021.254342.1304

Journal of
Applied Biotechnology Reports

Original Article

Evaluation of IgM-based ELISA for the Detection of Strongyloidiasis by NIE Protein

Katayoun Dastan [1*], Mehdi Assmar [1,2], Nour Amirmozafari [1,3], Fariborz Mansour Ghanaei [4,5], Mirsasan Mirpour [1]

[1] Department of Microbiology, Faculty of Basic Sciences, Lahijan Branch, Islamic Azad University, Lahijan, Iran
[2] Department of Parasitology, Pasteur Institute of Iran, Tehran, Iran
[3] Department of Microbiology, Faculty of Medicine, Iran University of Medical Sciences, Tehran, Iran
[4] Division of Gastroenterology, Faculty of Medicine, Guilan University of Medical Sciences, Rasht, Iran
[5] Gastrointestinal and Liver Disease Research Center, Razi Hospital, Rasht, Iran

Corresponding Author: Katayoun Dastan, PhD, Assistant Professor, Department of Microbiology, Faculty of Basic Sciences, Lahijan Branch Islamic Azad University, Lahijan, Iran. Tel: +98-9111430651, E-mail: K_dastan2009@yahoo.co.uk

Received October 25, 2020; **Accepted** March 1, 2021; **Online Published** March 7, 2022

Abstract

Introduction: Strongyloidiasis is a neglected disease caused by *Strongyloides stercoralis*. Fast and robust detection of this parasite can avoid its auto-infection cycle and therefore from the high parasitic load. The culture of stool and stool-based microscopy techniques are the most used methods for the parasite diagnosis; however, these methods are not sensitive enough. Immunological methods are more sensitive diagnostic tools and can be employed for the detection of this parasite. Since IgG4 immunoreactive protein (NIE) is a major pathogenicity factor of the parasite, in the present study, an IgM-ELISA method was developed to investigate the efficiency of anti-NIE IgM antibodies in the detection of *Strongyloides stercoralis*.

Materials and Methods: For this aim, 50 serum samples were gathered from positive patients along with 50 serum patients from healthy people. IgM antibodies were detected by using the ELISA technique and the data were analyzed by statistical analyses.

Results: Statistical analyses showed that IgM-ELISA was able to diagnose the disease with the sensitivity and specificity of 65.0%.

Conclusions: The developed IgM-ELISA method was relatively but not sufficiently successful at a robust diagnosis of strongyloidiasis. However, the sensitivity and specificity of this method were not good enough to be considered a reliable test for the diagnosis of the disease compared to the IgG- ELISA.

Keywords: Strongyloidiasis, IgM-ELISA, Immunodiagnosis, NIE protein, *Strongyloides stercoralis*

Citation: Dastan K, Assmar M, Amirmozafari N, Mansour Ghanaei F, Mirpour M. Evaluation of IgM-based ELISA for the detection of strongyloidiasis by NIE protein. J Appl Biotechnol Rep. 2022;9(1):557-562. doi:10.30491/JABR.2021.254342.1304

Introduction

Strongyloides stercoralis is responsible for about 100 million diseases each year.[1] This soil-transmitted helminth causes strongyloidiasis which is endemic mostly in tropical and subtropical areas.[2] The disease is reported from many areas in Iran, especially in the north of the country.[3,4] *S. stercoralis* has a special life cycle; following the exposure to contaminated soil, it can penetrate into the body through skin. It may be residing in the body without any clinical symptoms for years.[6] However, it can reach to lungs and gastrointestinal tract where it causes respiratory and diarrheal diseases, respectively. Sometimes, especially in immuno-compromised people, it causes complication diseases, such as hyper-infection and gastrointestinal ulcer disease.[5]

Nowadays, still there is no any gold standard test for the diagnosis of strongyloidiasis.[6] Stool culture is one of the mostly used methods for this purpose which has been used for decades; but, because of the low concentration of larva of *S. stercoralis* in the stool specimens, the sensitivity of this test is relatively low (30-50 %).[7] Moreover, the culture of stool has a risk of laboratory personnel contamination. To overcome these challenges, other methods have been developing.

In this regard, some serological tests have been developed. Because of the high sensitivity of these tests, that is higher than 70%, some of them are commercially available.[8] It should be noted, the specificity of these tests is a drawback; because of the possible cross-reactions with other parasites and long-term persistence of antibodies after an effective treatment. A recombinant antigen (NIE) has been used in order to increase specificity of serological methods such as ELISA and luciferase immunoprecipitation system (LIPS).[9,10]

NIE protein, which firstly introduced in 2002,[11] stimulates the release of histamine from basophils.[12] Antibody detection assays that exploited this antigen for the detection have had proper sensitivities (84%-98%) and specificities (95%-100%).[13]

In the present study, the applicability of anti-NIE specific IgM antibodies in the sera of human for the diagnosis of *S.*

Copyright © 2022 The Author(s). This is an open-access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

stercoralis was investigated. NIE protein was purified and used as the target antigen for the detection of specific IgM using indirect ELISA.

Materials and Methods

Samples

This prospective study was conducted among hospitalized patients infected with *S. stercoralis* in Rasht, Iran, for a period of one year, January 2018 to January 2019. The study was approved by the Ethics Committee of the Lahijan Branch, Islamic Azad University. Blood samples were collected from patients and centrifuged at 3000 rpm for 5 min to separate the serum and sera were stored at -20 °C for the analysis.

Preparation of NIE Protein

The preparation of NIE protein has been described elsewhere.[14] Briefly, the nucleotide sequence of *NIE* gene was adopted from GeneBank with the accession number of AAB97359. The gene was codon-optimized according to *E. coli* codon usage and synthesized in pET30b expression vector. The recombinant plasmid was transferred to competent *E. coli* BL21 (DE3) cells using heat-shock method.[15] The transformed cells were grown in LB broth media and the expression of the protein was induced by adding IPTG (with a final concentration of 1mM). The expression of the protein was analyzed by SDS-PAGE. Owing to its his-tag, the protein was purified by nickel column.[16]

IgM-ELISA Experiment

Determination of the Optimal Amount of Serum Required for the ELISA Reaction

ELISA test was performed to investigate if anti-NIE IgM is a proper test for diagnosis of *S. stercoralis*. For this purpose, the optimal amount of serum required for the ELISA reaction was determined. At this stage, the amount of coated antigen in each well was 4 micrograms and 50 different positive sera were mixed and used. The steps were as follows: 4 µg of NIE protein was dissolved in coating buffer (Na2CO3 1.59%, NaHCO3 2.93%, pH 9.8) and coated in each well for an overnight in 4 ºC. Then, the plate was washed with PBST (phosphate buffered saline containing tween). Then, to block the empty areas of antigen in the bottom of the wells (Blocking) that were not covered by antigen, 100 µl of PBST buffer containing 5% skimmed milk was added to each well and the plate was at 37 °C for 1 h. In the next step, the sera samples of patients were combined (50 samples), and a serial dilution was prepared (from dilution 1:100 to 1:3200) in PBST and added to the wells. No serum was added to one of the control wells containing the antigen, and 100 µl of the highest dilution (1:100) was added to the other well that lacked the antigen, and then the ELISA plate was placed at 37 °C for 1 h. Then, HRP-conjugated human anti-IgM antibody was added to each well and the plate was placed at 37 °C for 1 h. After that, OPD substrate solution (0.4 mg/ml OPD in 24 mM citric acid, 51 mM sodium phosphate, pH 5.0, 0.012% H_2O_2) was added to each well and finally the reaction was stopped with 2.5 M H_2SO_4 and using an ELISA reader, the absorbance at 492 nm was read.[17]

Determining the Minimum Concentration of Antigen for the Detection of Antibodies in Human Serum

To obtain the minimum concentration of antigen that can detect the presence of IgM antibodies in the serum, a serial dilution of antigen was prepared (4, 2, 1, 0.5, 0.25, 0.125, and 0.0625 µg). The microplate was then kept overnight at 4 °C. The ELISA steps were performed in accordance with the previous step. The difference is that step 4 (adding serum containing antibodies) was performed as follows: from the mixed positive sera, a 1:1000 dilution was prepared in PBST and added to the wells. No antibody was added to one of the control wells containing the antigen, and 100 µl of serum was poured into the other well without the antigen and then the ELISA plate was placed at 37 °C for 1 h.

Determination of IgM Titer in Positive and Negative Samples

To evaluate the ability of anti-NIE IgM antibodies to detect the infection with *S. stercoralis*, the titer of specific IgM antibody against NIE recombinant protein was measured in 20 positive and 20 negative samples separately.

Calculation of the Specificity and Sensitivity of the Method

The specificity and sensitivity of the method for the diagnosis of the infection with *S. stercoralis*, the following formula were exploited:[18]

Sensitivity = Number of true positives/Number of true positives + Number of false negatives
Specificity = Number of true negatives/Number of true negatives + Number of false positives

Statistical Analysis

The collected data were entered in GraphPad Prism version 8.0 (SanDiego, USA) and the sensitivities, specificities, positive predictive values, negative predictive values of the serological tests were calculated by this software. To compare the results, one-way ANOVA was used. The level of significance used was $p<0.05$. All experiments were done in triplicate.

Results

Preparation of NIE Protein

NIE is a 156 amino acid protein with the molecular weight of about 18 kDa. However, on a 12% SDSPAGE, NIE protein seems to have a molecular weight of 30-35 kDa. This phenomenon is related to the high GRAVY index and its low isoelectric point of NIE protein (this is completely

discussed in reference.[14] The expression of NIE protein as well as its purification was analyzed on a 12% SDS-PAGE, which the result can be seen in Figure 1. As seen in the Figure 1, the expression and purification of the protein has been done successfully. Following the purification, protein refolding was done by dialysis against phosphate buffer.

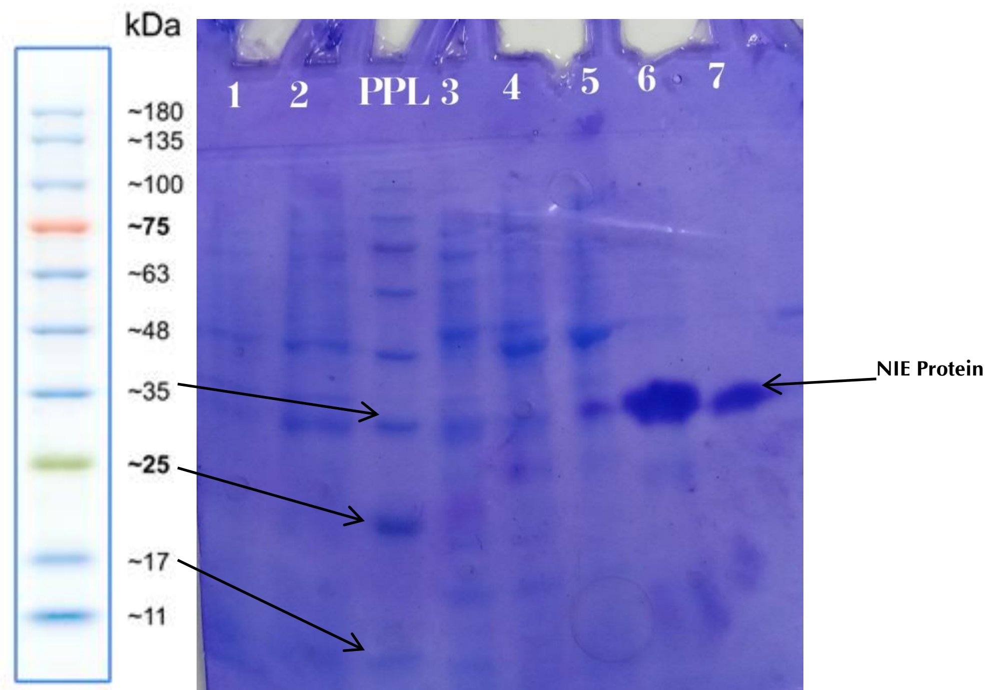

Figure 1. Expression Induction and Purification of NIE Protein. Lane 1: Before induction; Lane 2: After the induction with IPTG; PPL: Prestained Protein Ladder (SinaClone, Iran; Cat.No. SL7011); Lane 3: Flow through of the applying the expressed protein into the nickel column; Lane 4: Washing the column with urea buffer (pH 6.2); Lane 5: Washing the column with urea buffer (pH 5.9); Lane 6: Washing the column with urea buffer (pH 4.5); and Lane 7: Washing the column with MES buffer.

Determining the Optimum Sera Dilution and Minimum Concentration of Antigen for the Detection of Antibodies in Human Serum

To obtain the optimum sera dilution, we used 3 different dilutions of sera as controls: 1:100, 1:500, and 1:1000. The results showed that when 1:100 and 1:500 dilutions of patients' sera were used, a high titer of antibody was observed in all wells, including the control wells (wells without antigen but with serum). While, using a dilution of 1:1000 solved this problem (data are not presented), and for this reason, we used the same dilution to set up the test. Figure 2 shows the result of the experiment that was done to determine the minimum concentration of antigen for the detection of antibodies in human serum. As it seen in the Figure 2, all applied concentrations of the protein were able to detect IgM antibodies. However, the best result was seen when the protein concentration was 2 µg; so this concentration was used for other steps of this research.

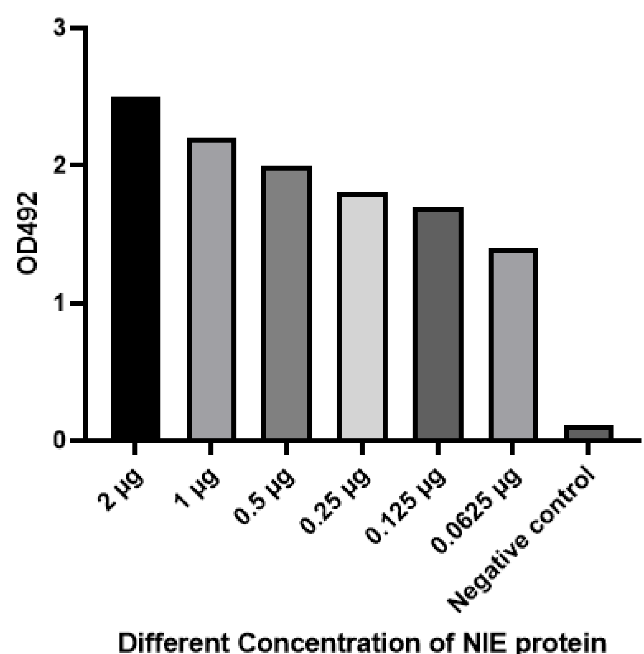

Figure 2. Investigating the Best Concentration of Protein for The Detection of IgM Antibodies in Human Sera. All applied concentrations of the protein are able to detect IgM antibodies. However, the best result was seen at 2 µg concentration of the protein.

Determination of IgM Titer in Positive and Negative Samples

To evaluate the ability of anti-NIE IgM antibodies for immunodiagnosis of infection with *S. stercoralis*, the titer of specific IgM antibodies against NIE recombinant protein was

measured in 20 positive and 20 negative samples separately. Tables 1 and 2 show the results of this experiment. As can be seen, a spectrum of ELISA results was obtained. To analyze the data, they were categorized in 6 different groups according to their ODs: lower than 0.4, from 0.4 to 0.6, from 0.6 to 0.8, from 0.8 to 1, from 1 to 2 and higher than 2 (Table 3). According to formulas 1 and 2, the sensitivity and specificity of the test were calculated (Table 4). As can be seen in Table 4, by the increase of Cut off, the sensitivity and specificity are decreased and increased, respectively. Considering 0.6 as the Cut off, the sensitivity and specificity obtained as 0.65.

Table 1. IgM Antibody Titers in 20 Positive Samples

	IgM Antibody Titers				
Samples	0.569	0.660	2.602	1.347	0.132
	0.980	0.744	0.510	0.744	2.602
	0.873	1.214	0.946	0.660	0.510
	0.789	0.365	0.472	0.472	0.948

Table 2. IgM Antibody Titers in 20 Negative Samples

	IgM Antibody Titers				
Samples	0.393	0.336	0.549	0.619	0.631
	1.676	0.362	0.441	0.670	1.066
	0.412	0.520	0.110	0.263	0.349
	0.758	0.912	0.342	0.537	0.351

Table 3. Classification of Raw Data from ELISA Test

IgM Titer	Patient	Healthy
0.4 or less	2	8
0.4-0.6	5	5
0.6-0.8	5	4
0.8-1	4	1
1-2	2	2
2-3	2	0

Table 4. The Specificity and Sensitivity of the Test Based on Each Cut Off

Cut off	Sensitivity (%)	Specificity (%)
0.4	95	30
0.6	65	65
0.8	35	85
1	20	90
≥2	10	100

Discussion

Strongyloidiasis is an endemic infectious disease prevalent in the north of Iran.[19] Robust and on-time detection of the infection with *S. stercoralis* is an urgent action, especially in immunocompromised people. Studies show the low sensitivity and specificity of the diagnostic methods for *S. stercoralis* detection. The asymptomatic nature of *S. stercoralis* and the limitations of diagnostic methods, leads to under-diagnosis. Due to the lack of a gold standard test for the diagnosis of the infection by this nematode, there have been many attempts in developing a fast and reliable test for this aim.[20,21] The diagnosis of this infection is generally performed by stool culture. However, because of the challenges relevant to this method, other tests have been developed.[22,23] Immuno-diagnostic tests have been widely used in this regard and ELISA-based methods have been promising detection methods in this regard.[24,25]

In the present study we investigated the applicability of the anti-NIE IgM antibodies for the diagnosis of strongyloidiasis and found that this method is relatively but not sufficiently appropriate method for this aim. According to our result, the best result was obtained when the cut off considered 0.6. In this situation both sensitivity and specificity were calculated as 65%. The previous studies on the immunodiagnosis of strongyloidiasis based on the detection of specific IgM and IgG have different sensitivity and specificity. The statistical analysis of the ROC curve is presented in the Table 5. As can be seen, the level below the chart is calculated as 0.7550. *P value* of the test is significant at the level of less than 0.05, which indicates that the performed laboratory test is able to distinguish somewhat between positive and negative samples.

For further investigation, a box diagram of the samples was also drawn (Figure 3). As can be seen in the Figure 3,

Table 5. The statistical Analysis of the ROC Curve

ROC Area	Results
Area Under the ROC Curve	
Area	0.7550
Standard Error	0.078
95% confidence intervals	0.6019 to 0.9081
P value	0.005817
Data	
Controls (Negative)	20
Patients (Positive)	20
Missing Controls	0

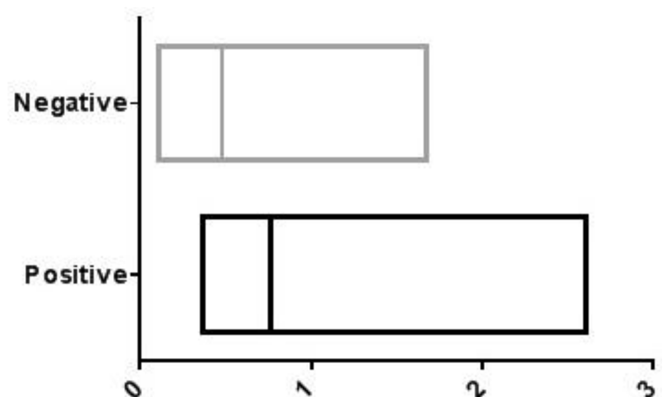

Figure 3. Box Plot of the IgM-ELISA Results.

the diagram of the positive samples was somewhat consistent with the diagram of the negative samples and the two diagrams overlap in part, which indicates the low ability of IgM to differentiate between the two groups (healthy and patient people).

Doorn et al., used four different ELISA-based serodiagnostic assays for the detection of anti-*S. stercoralis* antibodies in patients' sera, of which two assays were homemade and two assays were commercial kits. The sensitivities of the assays were between 83% to 93% and the specificity of their exploited assays ranged from 95.0% to 97.2%.[26] Ahmad et al., used ELISA to detect anti-*S. stercoralis* antibodies in patients' fecal samples. They reached to a detection sensitivity of 31.5 %.[27] Fradejas et al. compared the specificity and sensitivity of two commercial tests based on different antigens for *S. stercoralis* for the diagnosis of the helminth in humans. ELISA tests were based on crude larval suspension and NIE recombinant protein. The sensitivity of the ELISA test based on crude larval suspension ranged from 89.2% to 94.7% while the NIE-ELISA ranged from 72.3% to 78.9%. The specificity of the two assays was 72.3% to 89.3% for crude larval suspension-ELISA and 85.1% to 93.6% for the NIE-ELISA.[28] The detection of anti-NIE IgG antibodies (that we are focusing on) may be more appropriate method in this regard. It is rationale because IgG has a significant higher half-life than IgM.[29] Other studies have shown that IgG-based diagnosis of *S stercoralis* is high. For example, Krolewiecki et al., reached a sensitivity and specificity of 84% and 100%, respectively.[9] Pak et al., also reached 96.3% and 100% of sensitivity and specificity, respectively.[30]

Conclusion

The present study is the first report on the applicability of IgM antibodies for the detection of *S. stercoralis* based on NIE protein. In conclusion, the results show that the detection of anti-NIE IgM antibodies is somewhat able to differentiate between positive and negative strongyloidiasis samples, but this degree of differentiation is very low. In general, the sensitivity and specificity of this method were not good enough to be considered a reliable test for the diagnosis of the disease compared to the IgG- ELISA.

Authors' Contributions

K.D. and M.A. came up with the original idea and carried out the experiments. K.D. verified the methods and prepared the manuscript's draft. N.A. and F.M analyzed and verified the data and reviewed the final manuscript, supervised the findings of this work. All authors discussed the results and contributed to the final manuscript.

Conflict of Interest Disclosures

The authors declare that they have no conflicts interest.

Acknowledgment

This study was a part of K. Dastan's PhD thesis. We thank all staff of the Department of Microbiology of Islamic Azad University of Lahijan.

References

1. Jourdan PM, Lamberton PH, Fenwick A, Addiss DG. Soil-transmitted helminth infections. Lancet. 2018;391(10117): 252-65. doi:10.1016/S0140-6736(17)31930-X
2. Buonfrate D, Baldissera M, Abrescia F, Bassetti M, Caramaschi G, Giobbia M, et al. Epidemiology of *Strongyloides stercoralis* in northern Italy: results of a multicentre case–control study, February 2013 to July 2014. Eurosurveillance. 2016;21(31):30310. doi:10.2807/1560-7917.ES.2016.21.31.30310
3. Pagheh AS, Sharif M, Daryani A, Yazdani-Charati J, Nazar E, Asfaram S, et al. A cross-sectional analysis of intestinal parasitic infections among the general population in north of Iran. J Infect Dev Ctries. 2018;12 (2):120-6. doi:10.3855/jidc.9512
4. Asmar M, Ashrafi K, Amintahmasbi H, Rahmati B, Masiha A, Hadiani MR. Prevalence of intestinal parasitic infections in the urban areas of Bandar Anzali, northern Iran. J Guilan Uni Med Sci. 2014;22(88):18-25.
5. Zivari K, Yunina D, Grunwald M, Azar O, Tin K, Rahmani R. Esophagitis in Immunocompromised Patient from Strongyloides Reactivation. Am J Gastroenterol. 2019;114:S1023-4. doi:10.14309/01.ajg.0000596824.44656.14
6. Kalantari N, Chehrazi M, Ghaffari S, Gorgani-Firouzjaee T. Serological assays for the diagnosis of *Strongyloides stercoralis* infection: a systematic review and meta-analysis of diagnostic test accuracy. Trans R Soc Trop Med Hyg. 2020;114(6):459-69. doi:10.1093/trstmh/trz135
7. Anderson NW, Klein DM, Dornink SM, Jespersen DJ, Kubofcik J, Nutman TB, et al. Comparison of three immunoassays for detection of antibodies to *Strongyloides stercoralis*. Clin Vaccine Immunol. 2014;21 (5):732-6. doi:10.1128/CVI.00041-14
8. Bisoffi Z, Buonfrate D, Sequi M, Mejia R, Cimino RO, Krolewiecki AJ, et al. Diagnostic accuracy of five serologic tests for *Strongyloides stercoralis* infection. PLoS Negl Trop Dis. 2014;8(1):e2640. doi:10.1371/journal.pntd.0002640
9. Krolewiecki AJ, Ramanathan R, Fink V, McAuliffe I, Cajal SP, Won K, et al. Improved diagnosis of *Strongyloides stercoralis* using recombinant antigen-based serologies in a community-wide study in northern Argentina. Clin Vaccine Immunol. 2010;17(10):1624-30. doi:10.1128/CVI.00259-10
10. Ramanathan R, Burbelo PD, Groot S, Iadarola MJ, Neva FA, Nutman TB. A luciferase immunoprecipitation systems assay enhances the sensitivity and specificity of diagnosis of *Strongyloides stercoralis* infection. J Infect Dis. 2008;198(3):444-51. doi:10.1086/589718
11. Ravi V, Ramachandran S, Thompson RW, Andersen JF, Neva FA. Characterization of a recombinant immune-diagnostic antigen (NIE) from *Strongyloides stercoralis* L3-stage larvae. Mol Biochem Parasitol. 2002;125(1-2):73-81. doi:10.1016/S0166-6851(02)00214-1
12. *Strongyloides stercoralis* recombinant NIE antigen shares epitope with recombinant Ves v 5 and Pol a 5 allergens of insects. Am J Trop Med Hyg. 2005;72(5):549-53.
13. Rascoe LN, Price C, Shin SH, McAuliffe I, Priest JW, Handali S. Development of Ss-NIE-1 recombinant antigen based assays for immunodiagnosis of strongyloidiasis.

PLoS Negl Trop Dis. 2015;9(4):e0003694. doi:10.1371/journal.pntd.0003694
14. Dastan K, Assmar M, Amirmozafari N, Ghanaei FM, Mirpour M. Design, Expression and Purification of *Strongyloides stercoralis* IgG4 Immunoreactive Protein (NIE) in *Escherichia coli*. Iran J Parasitol. 2020;15(3):341-8. doi:10.18502/ijpa.v15i3.4198
15. Ebrahimi F, Ebadi V, Hajizadeh A, Tarverdizadeh Y, Bakhshi M. Designing a Recombinant Vaccine Containing Three Bacterial Proteins of EHEC, ETEC, and *shigella Dysentery* Antigens in *E. coli* and Evaluation of its Humoral Immunity in Mic. J Mazandaran Uni Med Sci. 2018;27(157):1-6.
16. Hajizade A, Salmanian AH, Amani J, Ebrahimi F, Arpanaei A. EspA-loaded mesoporous silica nanoparticles can efficiently protect animal model against enterohaemorrhagic *E. coli* O157: H7. Artificial Cells, Nanomedicine, and Biotechnology. 2018;46(sup3):S1067-75. doi:10.1080/21691401.2018.1529676
17. Hosseini SA, Nazarian S, Ebrahimi F, Hajizade A. Immunogenicity Evaluation of Recombinant *Staphylococcus aureus* Enterotoxin B (rSEB) and rSEB-loaded Chitosan Nanoparticles Following Nasal Administration. Iran J Allergy Asthma Immunol. 2020;19(2):159-71. doi:10.18502/ijaai.v19i2.2767
18. Lalkhen AG, McCluskey A. Clinical tests: sensitivity and specificity. Anaesth Crit Care Pain. 2008;8(6):221-3. doi:10.1093/bjaceaccp/mkn041
19. Shokri A, Sarasiabi KS, Teshnizi SH, Mahmoodi H. Prevalence of *Strongyloides stercoralis* and other intestinal parasitic infections among mentally retarded residents in central institution of southern Iran. Asian Pac J Trop Biomed. 2012;2(2):88-91. doi:10.1016/S2221-1691(11)60198-6
20. Formenti F, La Marca G, Perandin F, Pajola B, Romano M, Santucci B, et al. A diagnostic study comparing conventional and real-time PCR for *Strongyloides stercoralis* on urine and on faecal samples. Acta trop. 2019;190:284-7. doi:10.1016/j.actatropica.2018.12.001
21. Fernandez-Soto P, Celis-Giraldo CT, Collar-Fernandez C, Gorgojo Y, Camargo M, Mucoz J, et al. Strong-LAMP assay based on a *Strongyloides* spp.-derived partial sequence in the 18S rRNA as potential biomarker for Strongyloidiasis diagnosis in human urine samples. Dis Markers. 2020;2020:5265198. doi:10.1155/2020/52651 98
22. Kaewrat W, Sengthong C, Yingklang M, Intuyod K, Haonon O, Onsurathum S, et al. Improved agar plate culture conditions for diagnosis of *Strongyloides stercoralis*. Acta Trop. 2020;203:105291. doi:10.1016/j.actatropica.2019.105291
23. Nielsen PB, Mojon M. Improved diagnosis of *Strongyloides stercoralis* by seven consecutive stool specimens. Med Microbiol Infect Dis Virol Parasitol. 1987;263(4):616-8. doi:10.1016/S0176-6724(87)80207-9
24. de Faria LS, de Souza DL, Ribeiro RP, de Sousa JE, Borges IP, Avila VM, et al. Highly specific and sensitive anti-*Strongyloides venezuelensis* IgY antibodies applied to the human strongyloidiasis immunodiagnosis. Parasitology international. 2019;72:101933. doi:10.1016/j.parint.2019.101933
25. Conway DJ, Atkins NS, Lillywhite JE, Bailey JW, Robinson RD, Lindo JF, et al. Immunodiagnosis of *Strongyloides stercoralis* infection: a method for increasing the specificity of the indirect ELISA. Trans R Soc Trop Med Hyg. 1993;87(2):173-6. doi:10.1016/0035-9203(93)90477-8
26. Van Doorn HR, Koelewijn R, Hofwegen H, Gilis H, Wetsteyn JC, Wismans PJ, et al. Use of enzyme-linked immunosorbent assay and dipstick assay for detection of *Strongyloides stercoralis* infection in humans. J Clin Microbiol. 2007;45(2):438-42. doi:10.1128/JCM.01735-06
27. Ahmad AF, Hadip F, Ngui R, Lim YA, Mahmud R. Serological and molecular detection of *Strongyloides stercoralis* infection among an Orang Asli community in Malaysia. Parasitol Res. 2013;112(8):2811-6. doi:10.1007/s00436-013-3450-z
28. Fradejas I, Herrero-Martinez JM, Lizasoain M, Rodriguez de las Parras E, Perez-Ayala A. Comparative study of two commercial tests for *Strongyloides stercoralis* serologic diagnosis. Trans R Soc Trop Med Hyg. 2018;112(12):561-7. doi:10.1093/trstmh/try101
29. Jang WS, Kwak SY, May WL, Yang DJ, Nam J, Lim CS. Comparative evaluation of three dengue duo rapid test kits to detect NS1, IgM, and IgG associated with acute dengue in children in Myanmar. PLoS One. 2019;14(3):e0213451. doi:10.1371/journal.pone.0213451
30. Pak BJ, Vasquez-Camargo F, Kalinichenko E, Chiodini PL, Nutman TB, Tanowitz HB, et al. Development of a rapid serological assay for the diagnosis of strongyloidiasis using a novel diffraction-based biosensor technology. PLoS Negl Trop Dis. 2014;8(8):e3002. doi:10.1371/journal.pntd.0003002