BioBacta

Journal of Medical and Life Science
www.jmals.journals.ekb.eg

The validity of indirect haemagglutination assay (IHA) in the detection of

Schistosoma haematobium infection relative to microscopic examination

Ahmed Abdelhalim Yameny

Society of Pathological Biochemistry and Hematology, Egypt

Ahmed A. Yameny (Email: dr.ahmedyameny@yahoo.com)

ABSTRACT

Background : Several *schistosome* serodiagnostic assays have been developed over the last years, most of them are designed for the detection of anti-schistosomal antibodies which might remain for long periods of time after treatment, this study aimed to evaluate commercially available kit, indirect hemagglutination assay (IHA) as alternative to microscopic examination to diagnose *S.haematobium* infection **Subjects and methods:** This study is a cross-sectional study designed to evaluate an indirect hemagglutination assay (IHA), a commercially available kit was used for determine urinary schistosomiasis in the study population, this performed on 50 positive *S.haematobium* samples and 50 negative samples for individuals matching in age and gender, in addition, urine microscopic examination was done for detection of *S.haematobium* eggs by sedimentation centrifugation and nuclepore filtration techniques. **Results:** The results showed that an indirect hemagglutination assay (IHA) had a sensitivity of 42% and specificity of 80%. PPVs were 67.7% whereas NPVs were 57.97 %. As for diagnostic efficiency, it was 61%, where the area under the curve (AUC) was bad 0.58. **Conclusion and Recommendations:** Years ago this commercial kit which used in this study and also used in all medical laboratories in Egypt for detection of schistosome antibody in serum is called Fumouze bilharzial IHA Kit, this study showed low sensitivity (42%) and specificity (80%), that lower than expected results for detection of antibody and the kit is generally more expensive than microscopic examination for *S.haematobium* infection.

Keywords: *Schistosoma haematobium*, indirect hemagglutination assay, IHA, validity, urinary schistosomiasis.

----

1. Introduction

Schistosomiasis is considered a major cause of mortality and morbidity in Africa, the Middle East, South America, the Caribbean and Asia[1].Despite a steady decrease in the incidence of Schistosomiasis in Egypt according to the evolving schistosomiasis agenda 2017-2020, which aims to move from control toward final elimination[2]. Although the prevalence of *Schistosoma haematobium* infection decreased less than 0.2 % since 2016, there are more than 300 villages in Egypt with prevalence of more than 3%, which requires prolonged treatment and continuous examination [3]. Compared with direct proof of the presence of the eggs,

referred to as "parasitological diagnosis", serology provides a sensitive tool for the diagnosis of schistosomiasis, especially for infections with low intensity[4,5].

Concerninig immunodiagnosis of schistosomiasis which is based upon antibody detection, it is especially appropriate for testing travelers returning from disease-endemic areas, for population surveys in these areas, especially in those of low prevalence and low intensity and for determining the infection status of individuals not previously treated [6 -8]. Antibody-based immunodiagnosis of *Schistosoma* sp. infections is dependent upon the detection of parasite-specific antibodies. In turn, the sensitivity and specificity of the assay are dependent upon the antigen used to detect these antibodies. A variety of *Schistosoma* antigens, ranging from crude egg to adult worm preparations are used [9,10]. Light infections may rapidly stimulate high antibody levels. As such, they provide an excellent qualitative technique to demonstrate new cases of infection with very high sensitivity and specificity. On the other hand, antibody levels with the relative exception of IgG4 levels against egg antigens-are not quantitative indicators of the intensity of infection [11]. Antibody assays are therefore not well-suited for the diagnosis of active infection in endemic areas or for the follow-up of chemotherapy. Certainly in those cases where after therapy a few worms remain alive, antibody levels may remain elevated for prolonged periods. On the whole antibody assays, at the population level, they have proven to be more useful for measuring the serological status than for diagnosis [11]. However, if cheap, simple, sensitive and quantitative antibody assays were available they would be a useful adjunct to diagnose low endemic areas [12].

Serological tests which could, however, be used in routine clinical laboratories are an indirect hemagglutination assay (IHA) with adult *Schistosoma mansoni* worm antigens (WA) produced by Fumouze Laboratories (Levallois-Perret, France) (hereafter this assay is referred to as WA/IHA). It is used in most of the clinical laboratories in Egypt for quantitative detection of the antibodies found in sera from patients suffering from bilharziasis *Schistosoma manosoni* (intestinal location), with *Schistosoma haematobium* (urinary tract location) and with *Schistosoma intercalatum* (rectal location). The principle is based on indirect haemagglutination. Sensitized red blood cells are composed of sheep red blood cells coated with *Schistosoma mansoni* antigen. IHA not used in health center laboratories in Egypt but used in hospitals and private laboratories [13].

Diagnosis of human schistosomiasis is very central to individual case management and control programs, the continuation of research for the development of new diagnostic procedures or validation of the developed procedure is very crucial. Hence, the aim of the present study was to assess the diagnostic efficacy of a commercial indirect hemagglutination assay (IHA) for the detection of schistosomiasis haematobium relative to microscopic methods.

2. Material and methods

2.1. Study population and ethical consideration

This study included 100 patients attending the Ministry of health laboratory centers, in El-Fayoum Governorate, these study subjects were randomly selected irrespective of the age-group and both genders were included. All the studied population was informed about the purpose of sample collection and their consents were obtained. Patients were free to refuse sample collection, the project is approved by high institute of public health Alexandria university ethics Committee and ministry of the health district.

2.2. Study design

This research is a cross-sectional study designed to assess the diagnostic efficacy of a commercial indirect hemagglutination assay (IHA) for the detection of *Schistosoma haematobium* relative to microscopic methods in the study population. This study was targeting customers who came for laboratories of health centers for urine analysis. This

was performed on 50 positive *S.haematobium* samples and 50 negative samples for individuals matching in age and gender.

2.3. Collection and processing of urine samples

Clean specimen bottles were labeled with the needed information and issued to the participating individuals whose informed consent was sought earlier, each patient was given a wide mouth screw-capped container into which to void urine. This was carried out between 10.00 am and 2.00 pm when the ova count of *S.haematobium* is expected to be at its peak [14]. In this study, urinary schistosomiasis was defined as the presence of ova of *S.haematobium* in the urine

2.4. Urine microscopy

Urine samples were examined for the presence of *S.haematobium* eggs as in the sedimentation method of Cheesbrough (2006) [15]. Each urine sample was mixed thoroughly with a glass rod and two samples were taken each 10 ml urine, one sample for sedimentation centrifugation and another 10 ml urine sample for Nuclepore membrane filteration technique. The first 10 ml transferred into a centrifuge tube and centrifuged at 2000 rpm for 5 minutes at room temperature. The supernatant was then discarded and sediment transferred to a microscope glass slide and covered with a coverslip. A drop of Lugol's Iodine was added onto the coverslip prior to examination. The examination of the entire sediment was carried out using the x10 objective of a compound light microscope. The second 10 ml urine sample was examined using the

Nuclepore membrane filteration technique for *S.haematobium* eggs detection as in the method of Cheesbrough (2009) [16].

2.5. Blood sample:

1 ml blood sample was collected for performing Indirect haemagglutination test (IHAT Fumouze Diagnostics France) on 50 Positive *S.haematobuim* samples and 50 negative samples for individuals matching in age and gender.

2.6. Assay procedure as manufactory instructions:

Reagents of Fumouze IHAT kit and samples allowed to be at room temperature prior to testing, (serum was separated from a blood sample by centrifugation).

1- (1/40) stock-dilution of test serum: Delivered in a disposable tube: 0.05 ml of test serum and 1.95 ml of buffer solution.

2- Microplate reaction:

a- By means of a multi-channel micro pipettor, 50 ul of buffer solution was delivered into 8 wells, setting up the microplate widthwise.

b- 50 ul of serum stock-dilution(from step 1) was added in the first well, mixed with buffer and transferred preferably by means of a microdilutor 50 ul from the first well into the 2nd well, from the 2nd into the third and so on until the 6th well; 50 ul was discarded from the sixth well.

The following dilutions were obtained:

First well	second	third	fourth	fifth	sixth
1:80	1: 160	1:320	1:640	1:1280	1: 2560

50 ul of serum stock-dilution (from step 1) was added into the 7 th well, mixed with buffer and discard 50 ul. This 1:80 dilution constitutes "serum control" which serves for the control of natural anti-sheep agglutinins which may occur in certain sera.

c- Red blood cell suspensions carefully shook before use.

- one drop (16,66 ul) of sensitized red blood cells distributed in the first 6 wells.

- one drop (16.66 ul) of non-sensitized red blood cells added in the 7 th well (serum control).

- one drop (16,66 ul) of sensitized red blood cells added in the 8 th well which constitutes (reagent control), the role of which is the control of the validity of buffer and sensitized red blood cells.

- One "reagent control" was set up per microplate.

d- Wells content very carefully homogenized by lateral thrumming on the edges of the plate, placed flatwise (do not use orbital vibration). Read the reaction two hours later.

Reading of results:

Negative reaction: No haemagglutination: Presence of a more or less wide ring in the bottom of the well

Positive reaction: haemagglutination: no ring in the bottom of the well, sometimes, presence of a thin peripheral border, the titer is given by the first dilution showing a wide peripheral ring.
**Interpretation:**

- Titer less than 1: 160 the reaction is not significant of an evolutive infection. It may correspond with a past or an already treated infection, while titer equal to or above 1:160 significant reaction.

Fumouze schistosomiasis: reagent allows the detection of the antibodies present in sera from patients suffering from Bilharziasis with *Schistosoma mansoni* (intestinal localization), with *Schistosoma haematobium* (urinary tract localization), with *Schistosoma intercalatum* (rectal localization).

We consider the titer 1:160 as a positive result.
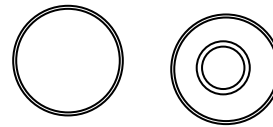
Positive   Negative

2.7. Stool microscopy:

Stool samples were examined for the presence of *S.mansoni* eggs, only negative stool samples for *S.mansoni* eggs of 100 population study samples were taken for that research study.

2.8. Statistical analysis

Results collected, coded, tabulated and analyzed through computer facilities using statistical methods *S.haematobium* infection was defined as any number of eggs greater than zero found in 10 ml of urine, was performed to compare with indirect hemagglutination assay (IHA) by commercially kit for Diagnosis of *Schistosoma haematobium* Infection, data analysis Correlation of the indirect hemagglutination assay (IHA) results with the gold-standard parasitological data was done using diagnostic accuracy tests. Sensitivity, specificity, positive and negative predictive values (PPV and NPV), likelihood ratios for positive results (LR+) and diagnostic efficiency and the area under the curve (AUC) were calculated. In this study, we considered using the sum of Nuclepore membrane filteration technique and Centrifugation sedimentation technique results as a gold standard to compare them with indirect hemagglutination assay (IHA) by commercially kit [17].

3. **Results**

Table (1): shows the diagnostic performance of indirect hemagglutination assay (IHA) by a commercially available kit as a diagnostic method for *Schistosoma haematobium* infection compared to microscopic examination techniques as the gold standard. In this study, we considered using the sum

of Nuclepore membrane filteration technique and Centrifugation sedimentation technique results as a gold standard[17].

The results showed that indirect hemagglutination assay (IHA) by a commercially available kit had a sensitivity of 42% and specificity of 80%. PPVs were 67.7% whereas NPVs were 58%. As for diagnostic efficiency, it was (61%), where the area under the curve (AUC) was bad (0.58).

Table (1): *S.haematobium* infection using an indirect hemagglutination assay (IHA) compared to microscopic examination techniques.

Test		Microscopic techniques		Total No.	Sensitivity %	Specificity %	PPV %	NPV %	PLR %	NLR %	Diagnostic Efficiency %	AUC
		Negative No .	Positive No .									
Indirect hemagglutination assay (IHA)	No	40	29	69	42	80	67.7	57.97	2.1	0.72	61	0.58
	Yes	10	21	31								
	Total	50	50	100								

4. Discussion

Patient schistosome infection is highly immunogenic, and anti-schistosome antibodies can be readily detected using a wide range of immunodiagnostic techniques [18]. Antibody-based assays are quite sensitive but it can not distinguish the history of exposure from active infection. Most assays have positive results for at least 2 years after cure and in many cases much longer [19]. They can also cross-react with other helminths and are not easily applicable under field conditions [20]. Such assays are important, however, for diagnosis in travelers, migrants, and other occasionally exposed people [21].

Immunodiagnostic techniques remain the best available methods for diagnosis in areas of low intensity of infection where the sensitivity and specificity of these methods appear to be satisfactory [22]. They can also be useful for incidence studies in children and in low-transmission or post-control settings [23].

Several *schistosome* serodiagnostic assays have been developed over the last years. Most of them are designed for the detection of anti-schistosomal antibodies which might remain for long periods of time after treatment [9]. Most routine techniques detect IgG, IgM or IgE against soluble worm antigen or crude egg antigen by EIA, indirect haemagglutination, or immunofluorescence. Seroconversion generally happens within 4-8 weeks of infection, but the interval can be as long as 22 weeks [24].

Imported cases of schistosomiasis in areas of non-endemicity are frequent among populations of immigrants and travelers returning from the tropics [25]. Most of these patients do not excrete eggs or excrete only a few eggs in an uneven fashion [26]. These cases are often unrecognized [27], and diagnosis of schistosomiasis by detection of specific antibodies is likely to be more sensitive than

diagnosis by the traditional microscopic techniques [28].

This study showed that commercially available IHA produced by Fumouze laboratories has a low sensitivity 42%, specificity 80% and positive predictive value was 67.7% with kappa coefficient = 0.22 (fair), and AUC 0.58 which is bad, while using circulating antigen in urine(CAg) has more sensitivity 56% and sufficient AUC 0.63[29].

Indirect Haemagglutination (IHA) tests using adult worm antigens were previously evaluated for the diagnosis of schistosomiasis by a few investigators [30]. With a few exceptions such as the ones used by van Gool et al. [31], most of the previously used IHA test kits were not commercially available for use in an epidemiologic applications. These previous works have reported sensitivity ranging from 71 to 100% and specificity ranging from 80 to 100% [31].

(Berhanu Erko et al., 2009) recorded that the sensitivity of IHA test for the diagnosis *Schistosomiasis mansoni* was found to be 83% and this, while the specificity was lower (53%) they used A commercial IHA kit (Cellognost Schistosomiasis H) manufactured by Dade Behring Marburg GmbH, Germany, and the test was performed following procedures given by the manufacturer [32].

(Tom van G et al., 2002) evaluate the same commercial kit IHAT produced by fumouze laboratories for detection of *Schistosoma haematobium*, the sensitivity of the study was 80% and the specificity was 98.9% the study considered cut off 1/160 and carried only on 25 cases infected with *Schistosoma haematobium* [31], while Kinkel et al.,(2012) found that the sensitivity of IHA for *S. haematobium* was 71.4% and specificity was 99.0% [33].

The present study carried on 50 infected cases with *Schistosoma haematobium* and 50 negative cases with *schistosoma haematobium* nor *schistosoma mansoni* for 50 negative cases beside

sedimentation, nuclepore filteration and made kato method for stool to be sure the cases negative also for *schistosoma mansoni*. The present study showed low sensitivity 42% below the range of previous studies with range for 71% to 100% and the specificity was 80% meanwhile the range of previous studies ranged from 80% to 100%.

Conflict of interest

There are no conflicts of interest.

Financial support and sponsorship

5. References:

1. Ortu G, O. Ndayishimiye, M. Clements, D. Kayugi and C. Campbell Jr. et al., 2017. Countrywide reassessment of Shistosoma mansoni infection in Burundi a urine-circulating cathode antigen rapid test: Informing the national control program. Am. J. Trop. Med. Hyg., 96:664-673.

2. Ahmed A. Yameny. The evolving schistosomiasis agenda 2017- 2020 in Egypt: Moving from control toward final elimination. J Biosci App Res .2017;3(2): 48- 54.

3. Ahmed A. Yameny. Schistosomiasis haematobium Prevalence and Risk factors in EL-Fayoum Governorate, Egypt. J Biosci App Res .2017;3(4): 191- 201.

4. K.G. Weerakoon, G.N. Gobert, P. Cai, D.P. McManus, Advances in the diagnosis of human schistosomiasis, Clin. Microbiol. Rev. 28 (4) (2015) 939e967.

5. E.M. Dawson, J.C. Sousa-Figueiredo, N.B. Kabatereine, M.J. Doenhoff,J.R. Stothard, Intestinal schistosomiasis in pre school-aged children of Lake Albert, Uganda: diagnostic accuracy of a rapid test for detection of antischistosome antibodies, Trans. R. Soc. Trop. Med. Hyg. 107 (10) (2013) 639e647.

6. Maddison SE. The present status of serodiagnosis and seroepidemiology of schistosomiasis. Diagnostic microbiology and infectious disease. 1987; 7: 93-105.

7. Noya AZ, Fermin B, Losada A, Colimenares C and Hermoso T. Humoral immune response of children with chronic schistosomiasis. Isotype recognition of adult worm antigens. Paraiste immunology 1995; 17: 319-328.

8. Certon MSL, Chitsulo J, Sullivan J, Pilcher M, Wilson J, Noh VC, et al. Schistosomiasis and lake Malawi: Transmission and risk to expatriates and Tourists. Lancet 1996; 348: 1274-1278.

9. Mott KE and Dixon. Collaborative study on antigens for immunodiagnosis of schistosomiasis. Bulletin of the World Health Organization 1998; 60: 729-753.

10. Tsang K and Wilkins. Immunodiagnosis of schistosomiasis: screen with fast-ELISA and confirm with immunoblot. Clinics in Laboratory Medicine 1991; 11: 1029-1039.

11. WHO (1998): Report of the WHO informal consultation on schistosomiasis control, Geneva WHO/ CDS/ CPC/ S1P/ 1999. 2.

12. WHO (2000): Report of the WHO informal consultation on schistosomiasis in low transmission areas: control strategies and criteria for elimination, London (unpublished document) WHO/ CDS/ CPE/ SIP/ 2001. 1.

13. Ahmed A. Yameny. Evaluation of Health Centers Laboratory Results for *Schistosoma haematobium* Infection in El-Fayoum Governorate, Egypt. J Biosci App Res .2016;2(12): 788- 796.

14. Eyong ME, Ikepeme EE, Ekanem EE. Relationship between *Schistosoma haematobium* infection and urinary tract infection among children in South Eastern, Nigeria. Niger Postgrad Med J(2008);15:89‑93.

15. Cheesbrough M. District laboratory practice in Tropical countries part 2. United Kingdom :Cambridge University Press. Cambridge. 2006; 323-41.

16. Cheesbrough M. Laboratory-practice-in-tropical-countries-part-1. United Kingdom :Cambridge University Press. Cambridge. 2009;p.357

17. Ahmed A. Yameny. Evaluation of Nuclepore Filteration Technique for Diagnosis of *Schistosoma haematobium* Infection.J Biosci App Res. 2015;1(6):335-341.

18. Doenhoff MJ, Chiodini PL, Hamilton JV (2004) Specific and sensitive diagnosis of schistosome infection: can it be done with antibodies? Trends Parasitol 20: 35-39.

19. Rabello AL, Garcia MM, Pinto da Silva RA, Rocha RS, Katz N. Humoral immune responses in patients with acute Schistosoma mansoni infection who were followed up for two years after treatment. *Clin Infect Dis* 1997; **24:** 304–08.

20. Rabello A. Diagnosing schistosomiasis. *Mem Inst Oswaldo Cruz*1997; **92:** 669–76.

21. Bottieau E, Clerinx J, De Vega MR, et al. Imported Katayama fever: clinical and biological features at presentation and during treatment. *J Infect* 2006; **52:** 339–45.

22. Goncalves MM, Barreto MG, Peralta RH, Gargioni C, Goncalves T, et al. (2006) Immunoassays as an auxiliary tool for the serodiagnosis of *Schistosoma mansoni* infection in individuals with low intensity of egg elimination. Acta Trop 100: 24-30.

23. Noya O, de Noya BA, Losada S, et al. Laboratory diagnosis of schistosomiasis in areas of low transmission: a review of a line of research. *Mem Inst Oswaldo Cruz* 2002; **97:** 167–69.

24. Jones ME, Mitchell RG, Leen CL. Long seronegative window in schistosoma infection. *Lancet* 1992; **340:** 1549–50.

25. Whitty CJ, Mabey DC, Armstrong M, Wright SG, and Chiodini PL. 2000; Presentation and outcome of 1107 cases of schistosomiasis from

Africa diagnosed in a non-endemic country. Trans.R. Soc. Trop. Med. Hyg. 94: 531-

26. Ye XP, Donnelly CA, Anderson RM, Fu YL, and Agnew M.1998. The distribution of schistosoma japonicum eggs in faeces and the effect of stirring faecal specimens. Am.Trop. Med. Parasitol. 92; 181 – 185.

27. Ebrahim A, El Moshedy H, Omer E, El Daly S, and Barakat R. 1997; Evaluation of the Kato-Katz thick smear and formal ether sedimentation techniques for quantitative diagnosis of schistosoma mansoni infection. Am.J. Trop. Med. Hyg. 57: 706 – 708.

28. Hamilton JV, Klinkert M, and Doenhoff JM. 1998; Diagnosis of schistosomiasis: antibody detection, with notes on parasitological and antigen detection methods. Parasitology 117: S41 – S57.

29. Ahmed A. Yameny. Evaluation of circulating antigen in urine technique for diagnosis of *Schistosoma haematobium* infection. J Biosci App Res .2018; 4(4): 519- 525.

30. Gui M, Idris MA, Shi YE, Muhling A, Ruppel A, Reactivity of *schistosoma japonicum* and S.mansoni antigen preparations in indirect haemagglutination (IHAT) with sera of patients with homologous and heterologous schistosomiasis .Ann Trop Med Parasitol 1991; 85: 599 – 604.

31. Van Gool T, Vetter H, Vervoort T, Doenhoff MJ,Wetsteyn J, Overboosch D. Serodiagnosis of imported schistosomiasis by a combination of a commercial Indirect Hemagglutination Test with *Schistosoma mansoni* adult worm antigens and an Enzyme-Linked Immunosorbent Assay with S.mansoni eggs antigens. J Clin Microbiol 2002; 40: 3432 – 3437.

32. Erkol B, Legesse1M, Balcha1F, Medhin1G, Sori2T. Diagnosis efficacy of Indirect Hemagglutination Test in relation to kato method for diagnosis of schistosomiasis mansoni Ethiop J Health Sci. July 2009 vol.19, No.2

33. Kinkel H.F., Dittrich B. Baumer and T Weitzel, 2012. Evaluation of eight serological tests for diagnosis of imported Schistosomiasis. Clin Vaccine Immunol, 19:948-953.