JHIPH
Journal of High Institute of Public Health 2018;48(1):18-23.

Original Article

Validity of PCR Versus Coproscopic Examination for Diagnosing Infection with Schistosoma mansoni in a Low Intensity Endemic Area in Egypt

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Abstract

Background: Egypt is a Schistosoma endemic country with localized foci of transmission. Although ongoing public health control programs could reduce infection rates, yet the situation evaluation necessitates a good diagnostic technique.
Objective(s) As the current parasitological methods lack efficiency, the aim of present study was to assess the validity of conventional polymerase chain reaction (PCR) versus coproscopic techniques as gold-standard to diagnose *S. mansoni* infection in a low intensity endemic area in Egypt.
Methods: A cross sectional study was used to examine faecal samples of 120 primary schoolchildren; 74 (61.7%) were males and 46 (38.3%) were females with mean age of 10.16±0.798 years (range: 9-12 years). Three fresh stool samples were collected on three consecutive days from each study subject and examined by formalin-ether concentration (FEC); three slides from one fecal sample of the first day. Ten slides from three fecal samples on three consecutive days were examined by Kato-Katz technique; 6 slides on the first day and 4 slides from 2nd and 3rd day samples, 2 each. Lastly, one sample of the fecal specimen of the 1st day was tested by PCR.
Results: *S. mansoni* infection prevalence was 40.0 %, 69.2% and 80.8% by FEC, Kato-Katz, and PCR techniques, respectively. All infected cases were of low intensity infection (<100 epg). The overall geometric mean egg count (GMEC) was 13.35 and 33.34 epg stool by FEC and Kato-Katz techniques, respectively. The Kappa index (0.75) and diagnostic parameters showed a good diagnostic value of PCR as compared to copromicroscopic examination.
Conclusion: PCR demonstrated a good diagnostic performance for the detection of *S. mansoni* in low intensity endemic area versus coproscopic examination as gold-standard.

Keywords: Diagnostic performance; formalin-ether concentration; Kato-Katz; PCR; *Schistosoma mansoni*

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Suggested Citations: Bassiouny HK, Eassa SM, Hazzah WA, El-Komy EO, Barakat R. Validity of PCR versus coproscopic examination for diagnosing infection with *Schistosoma mansoni* in a low intensity endemic area in Egypt. JHIPH. 2018;48(1):18-23.

INTRODUCTION

Egypt is an endemic country for schitosomiasis with localized transmission foci. Although public health control programs have been ongoing, reducing infection rates, re-infection is continuing to be a problem in rural areas due to poor sanitary conditions. To optimize the implementation of a control strategy in a particular context, a simple monitoring program should be in place that can provide feedback and reinforcement to those who are actually doing the work.[1] The available diagnostic methods are far from ideal. The microscopic demonstration of eggs in stool either by Kato-Katz[2] or formalin-ether concentration (FEC) technique[3] lack sensitivity in low intensity and post-treatment situations. Therefore, it is necessary to increase the number of stool samples or number of slides prepared from a single stool sample[4] to overcome the lack of sensitivity of the parasitological methods. Other diagnostic alternatives include immunologic methods such as detection of parasite-specific antibodies and circulating antigens. A commercially available urine-dipstick which detects schistosome circulating cathodic antigen (CCA) in host urine is being increasingly applied.[5,6] The detection of circulating

antigens is a highly specific assay and proof to be more sensitive than the detection of eggs in areas of low endemicity. Moreover, the antibody detection assays have been shown to be more sensitive than the parasitological examination[7] but generally lack specificity.[8] In an attempt to surpass these diagnostic limitations, polymerase chain reaction (PCR) has been described for detecting *S. mansoni* DNA in human fecal samples. The amplification reaction can detect as little as 1 fg of a highly repetitive *S. mansoni* DNA sequence, showed no cross- reactivity with other related helminthes and was 10 times more sensitive than Kato-Katz technique.[9]

The elegant published meta-analysis study highlights the need for more investigations on different methods for detecting schistosomiasis in preschool-aged children. Thereupon, it is crucial to incorporate additional sensitive diagnostic tools to determine infection level among those with light infection.[10]

PCR-based diagnostics are not commonly used for clinical diagnosis within *Schistosoma*-endemic countries, although highly specific and sensitive, because they require expensive laboratory equipment and highly skilled personnel.[11,12] Currently there is a paucity of studies which evaluate PCR in community settings in Egypt. Thus, we aimed in the present study to evaluate the validity of conventional PCR versus coproscopic examination for the diagnosis of *S. mansoni* infection in a low intensity endemic area in Egypt, to be further introduced as a diagnostic tool in most laboratories and to policy makers for community based control programs.

METHODS

Study area: The study was carried out in Arab El-Mahdar village, Kafr El-Sheikh Governorate, Egypt. The village is situated in the northern part of the Nile Delta bounded by Rosetta branch of the Nile River in the West and the Mediterranean Sea in the North, figure 1. The total population was of 3570 individuals. The study area was selected since most of the recorded cases of *S. mansoni* infections either originated from that village or in the surrounding areas. Due to inadequate water supply, inhabitants of the village depend mainly on public taps for drinking and cooking while canal water is used for irrigation and other domestic activities as washing utensils or clothes.

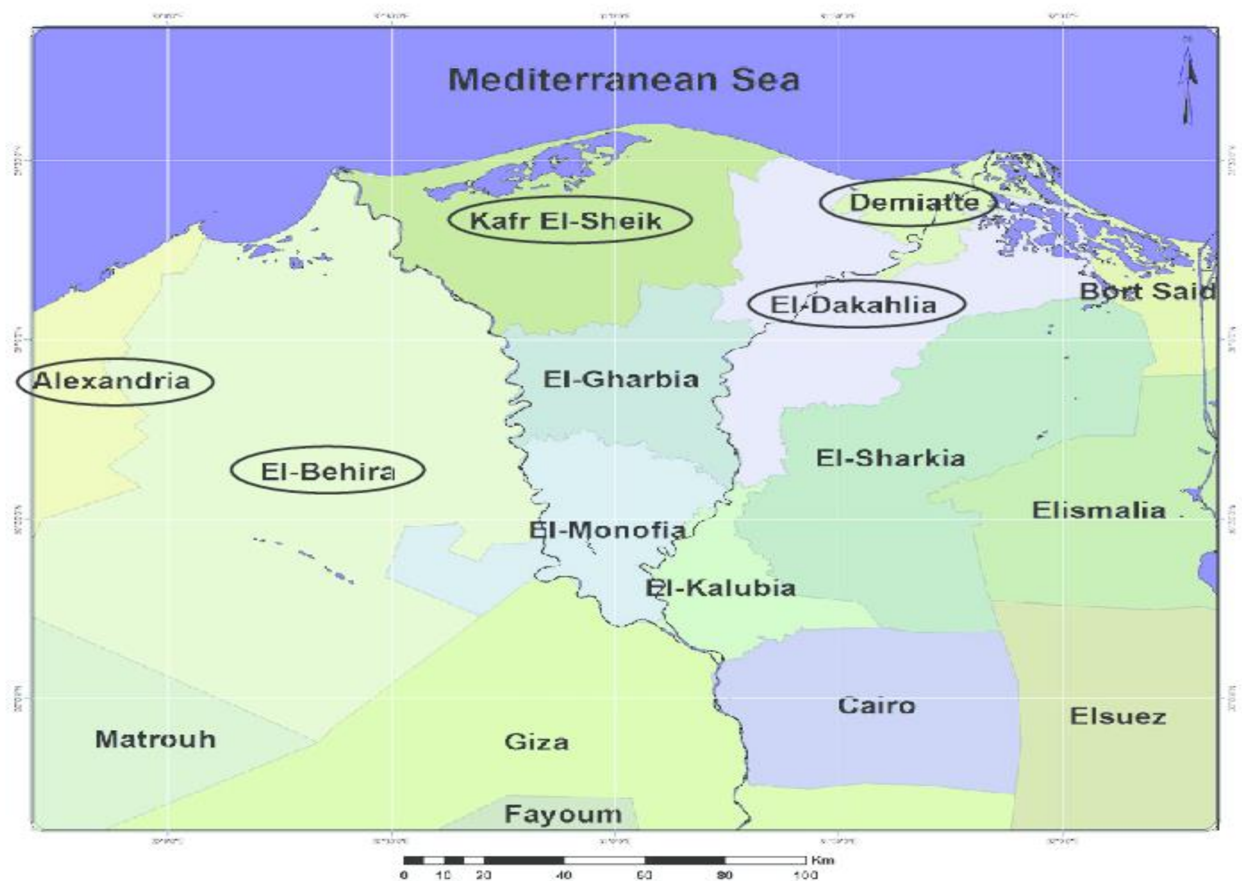

Figure 1: The Nile Delta Governorates

Children use canal water for recreation especially during summer season. The village lacks sanitary sewage disposal system. Domestic excreta are disposed of through septic tank in some houses, while those located in the vicinity of canal water pour their excreta directly into the nearest water stream. The majority of the inhabitants are farmers and anglers.

Study population: A cross sectional approach was used. Three classrooms of the 3rd grade and two classrooms of the 4th grade were randomly selected from Arab El-Mahdar primary mixed school. Children who had no history of antihelmenthics or antischistosomal drugs administration in the last month prior to screening were included in the study. Out of 165 children participated in the study, 120 children [74 (61.7%) males and 46 (38.3%) females] with mean age 10.16±0.798 years (range: 9-12 years) were able to give three stool samples on three consecutive days with a compliance rate 72.73%.

Stool samples collection and analysis: Three fresh morning stool samples were collected on three consecutive days from each child. The samples were examined by different techniques as follows:

- Kato-Katz technique[2]: a total of 10 slides from 3 fecal samples; 6 slides on the first day and 4 slides from the 2nd and 3rd day samples, 2 each.
- Formol ether concentration (FEC) technique[3]: 1 g stool was used to prepare three slides from the 1st day fecal sample.
- PCR: one sample of the fecal specimen of the first day was examined by PCR. DNA was extracted from 200 mg stool sample using Mini Kits - supplied by iNtRON Biotechnology, Inc. Germany (Cat. #17451)- according to the manufacturer's instructions. The primers described by Hamburger *et al.,*[14] were used for amplifying the 121-bp tandem repeat DNA sequence of *S. mansoni*. The amplification reaction was performed according to Pontes et al.,[9] and the amplified product was separated on agarose gel electrophoresis.

The multiple slides examination in the present study was followed according to the recommendation of Enk *et al.,* [15] to increase the sensitivity of the parasitological techniques.

Statistical Analysis

Since a gold standard test is not available for the detection of parasitic infections, the combined results from the two methods, Kato-Katz (10 slides) and FEC (3 slides) techniques, were used as a diagnostic gold standard.[16] Correlation of the diagnostic parameters of PCR versus coproscopic techniques was done using diagnostic accuracy tests; sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and Receiver operating characteristic curves (ROC) analysis.[17] Cohen's Kappa coefficient was used for evaluation of agreement of diagnostic methods. P value less than 0.05 was considered significant. EPG values were transformed into log 10 of the EPG +1 to allow for the zero counts and the GMEC was computed as the anti-log 10 of the mean of the log10 egg count.

Ethical Considerations

The Ethics Committee of the High Institute of Public Health, Alexandria University, approved the study protocol. An oral informed consent was obtained from the school officials and the parents of the children who participated in the study after explaining the objective of the work. Children infected with different parasitic infections (as *Trichuris trichiura, Enterobius vermicularis, Ascaris lumbricoides*) were treated with albendazole and those infected with *S. mansoni* were treated with praziquantel according to the WHO guidelines. [13]

RESULTS

The prevalence of *S. mansoni* infection was 40.0%, 69.2%, 80.8% by FEC, Kato-Katz and PCR techniques, respectively. Cohen's Kappa coefficient (k) = 0.687. (i.e. good agreement between the Kato Katz technique (10 slides) and FECS technique (3 slides) for the diagnosis of *S. mansoni* infection was found. The overall geometric mean egg count (GMEC) was 13.35 and 33.34 epg stool by FEC and Kato-Katz techniques, respectively (Table1).

Table 2 illustrates that all cases reported positive by coproscopic examination were also positive by PCR. In addition, 11 pupils were positive only by PCR who were negative by coproscopic examination.

Table 1: Distribution of *S. mansoni* infected cases according to the diagnostic methods used and GMEC

Diagnostic Technique	Examined sample (n=120)		Percent infected (%)	GMEC (Epg stool)
	Positive	Negative		
FEC Technique (3 slides)	48	72	40.0	13.35
Kato-Katz Technique (10slides)	83	37	69.2	33.34
conventional PCR	97	23	80.8	-

FEC Technique, Formol- ether concentration sedimentation technique; GMEC, Geometric mean egg count; Epg stool, eggs per gram stool

Cohen's Kappa coefficient (k) was 0.750 indicates a good agreement between PCR and coproscopic examination as a diagnostic gold- standard. Table 3 depicts the diagnostic parameters of PCR versus coproscopic examination. The sensitivity of 100%, specificity of 67.6%, PPV of 88.7%, and NPV of 100% with an overall diagnostic efficiency of 90.8% was calculated. The likelihood ratio for positive results (PLR) of 7.82, the likelihood ratio for negative results (NLR) of 0, discrimination ability (DA) of 88.70% and the area under the curve (AUC) of 0.838 were also elaborated, (Figure 2).

Table 2: Agreement between the PCR technique and the diagnostic gold-standard for the diagnosis of *S. mansoni* infection

		Diagnostic gold-standard*		
		Negative	**Positive**	**Total**
Conventional PCR	Negative	23	0	23
	Positive	11	86	97
	Total	34	86	120

Cohen's Kappa coefficient (k) = 0.750. (Good agreement)
*Diagnostic gold-standard=combined results of the stool examination by both Kato-Katz (10 slides) and FECS (3 slides) techniques.

Table 3: Diagnostic parameters of PCR versus coproscopic examination as a diagnostic gold standard*

DE %	Sensitivity % (95%Cl)	Specificity % (95%Cl)	PPV % (95%Cl)	NPV % (95%Cl)	PLR (95%Cl)	NLR (95%Cl)	DA %
90.8	100 (94.6- 100)	67.6 (49.4- 82.0)	88.7 (80.2- 93.9)	100 (82.2-100)	7.82 (4.46-13.7)	0 (0-Non)	88.70

DE, Diagnostic efficiency; PPV, Positive predictive value; NPV, Negative predictive value; PLR, likelihood ratio for positive results; NLR, likelihood ratio for negative results; DA, Discrimination ability; AUC Area under the curve; CI, confidence interval

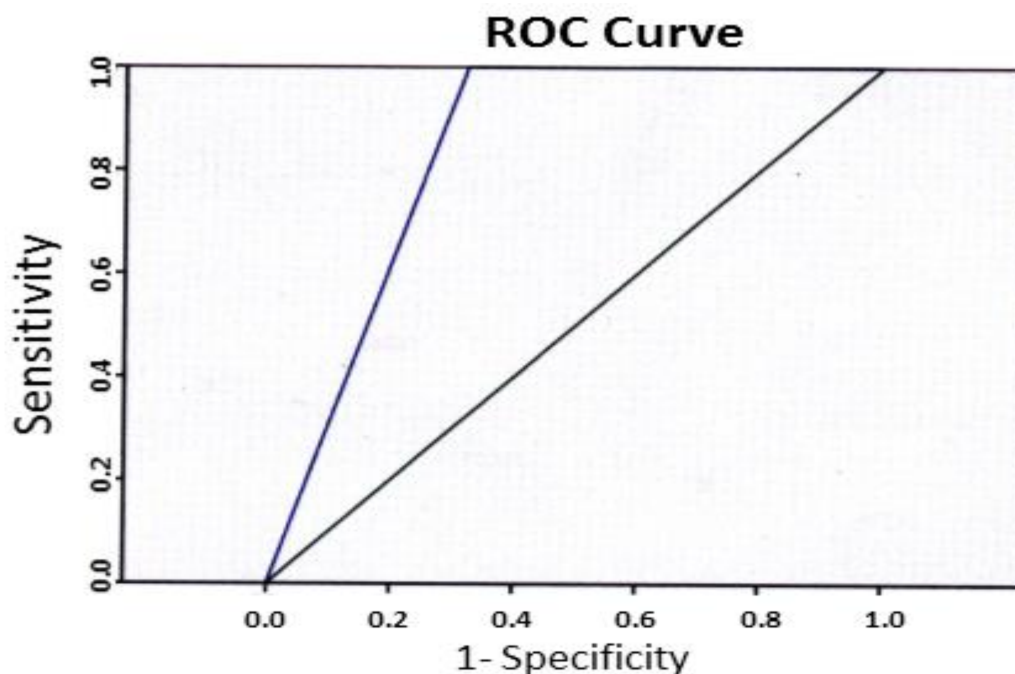

Figure 2: ROC curve and the AUC of cPCR for the diagnosis of *S. mansoni* infection compared to microscopic examination as a gold-standard technique

DISCUSSION

The present findings have demonstrated that out of 120 studied children, 97 (80.8%) were infected cases detected by PCR compared to 48 (40.0%) and 83 (69.2%) cases detected by FEC and Kato-Katz techniques, respectively. These findings were previously confirmed by many authors in endemic areas.[18,19] In addition, all the infected cases were of low intensity category and Cohen's Kappa coefficient indicated a good agreement between PCR and copromicroscopic examination. Furthermore, analysis of the current results indicated that 86 cases were positive by gold standard and 97 cases were diagnosed by PCR technique. These 11 false negative cases were reported by copromicroscopic examination and confirmed positives by PCR could be explained to be due to 2 main reasons: first, all 11 cases are actually infected but their intensity of infection is too low to be detected by Kato-Katz technique. Pontes et *al.,* (2002) demonstrated that PCR technique was able to detect *S. mansoni* DNA in stool samples containing 2.16 epg stool which couldn't be detected by Kato-Katz method. [9] The high sensitivity of PCR was attributed to unusual high copy number (600,000/cell) of target sequence which comprises at least 12% of *S. mansoni* genome that enables the detection of fraction of a single *S. mansoni* individual cell instead of entire eggs essential for microscopic examination.[20] Overall, PCR can be an important tool for detecting *S. mansoni* infection in individuals excreting few eggs in feces.

The second reason is these 11 infected cases could be in the prepatent period of *S. mansoni* infection where PCR was able to detect the circulating DNA of *S. mansoni* starting from the 3rd day post infection (P.I.) before the eggs were detected in the faeces in the 7th week P.I. This hypothesis was documented by Hussein et al., who evaluated the PCR technique for detection of free circulating *S. mansoni* DNA in serum in the early prepatent period in experimentally infected mice, in comparison to the commonly used indirect hemagglutination assay (IHA) for the detection of bilharzial antibody and stool examination. Their results showed that the earliest deposition of eggs in the small intestine was observed at the 5th week P.I. and the eggs were detected in faeces in the seventh week P.I. PCR detected free circulating DNA of *S. mansoni* starting from the third day P.I., while IHA failed to detect infection up to the eighth week P.I. [21]

It is concluded that detection of free circulating DNA by PCR can be used as a valuable test for early diagnosis of prepatent *S. mansoni* infection. This finding was also confirmed by other authors.[22] In addition, the obtained results of the diagnostic parameters of the PCR versus coproscopic examination as gold- standard revealed that PCR had the sensitivity of 100% indicating that PCR was able to detect all the school children who were infected by *S. mansoni*. The specificity of PCR was relatively high (67.6%). The PPV, which indicates the probability of having schistosomiasis among children with positive PCR was high (88.7%). This means that 11.3% of children didn't have schistosomiasis when their PCR results were positive. NPV, which indicates probability of not having schistosomiasis among children with negative PCR, was 100%. This means that none of the children with negative PCR had the probability of having the disease. Similar results were also reported by other authors.[20,23]

The current study also revealed that the PLR was 7.82 which indicated that the PCR was about 8 times more likely to detect those who were infected by *S. mansoni*, than those diagnosed by coproscopic examination as gold-standard. The NLR of 0 indicated that PCR was a perfect test for diagnosing low intensity infection i.e. the effect size is large. By performing the ROC curve and estimating the AUC to be 0.838 indicated an excellent diagnostic parameter. In addition, the DA of 88.70% indicated that PCR had diagnostic efficiency of 90.8% i.e. good diagnostic performance. We emphasize on the importance of using sensitive diagnostic methods to improve accuracy in estimating true infection prevalence as this has implications on the required MDA regimen for the population. In the present study, PCR was highlighted as a valuable sensitive diagnostic tool that could be applied in conjunction with the parasitological technique.

CONCLUSION AND RECOMMENDATIONS

The present study has documented that conventional PCR technique is of good performance in detecting *S. mansoni* DNA in stool samples of infected individuals with low worm burden as compared to coproscopic examination as gold-standard.

Conflict of Interest: None to declare.

REFERENCES

1. UNICEF/UNDP/World Bank/WHO TDR. Community-directed Interventions for Major Health Problems in Africa. A Multi-Country Study. Final Report. Geneva: World Health Organization. 2008. 132p.
2. Katz N, Chaves A, Pellegrino J. A simple device for quantitative stool thick-smear technique in Schistosomiasis mansoni. Rev do Inst Med Trop São Paulo. 1972;14(6):397–400.
3. Ritchie LS. An ether sedimentation technique for routine stool examinations. Bull US Army Med Dep. 1948 Apr;8(4):326.

4. Rabello A, Pontes LA, Dias-Neto E. Recent advances in the diagnosis of *Schistosoma* infection: the detection of parasite DNA. Mem Inst Oswaldo Cruz. 2002 Oct;97:171–2.
5. Standley CJ, Lwambo NJS, Lange CN, Kariuki HC, Adriko M, Stothard JR. Performance of circulating cathodic antigen (CCA) urine-dipsticks for rapid detection of intestinal schistosomiasis in schoolchildren from shoreline communities of Lake Victoria. Parasit Vectors. 2010 Jan;3(1):7.
6. Tchuem Tchuenté L-A, Kueté Fouodo CJ, Kamwa Ngassam RI, Sumo L, Dongmo Noumedem C, Kenfack CM, et al. Evaluation of circulating cathodic antigen (CCA) urine-tests for diagnosis of *Schistosoma mansoni* infection in Cameroon. PLoS Negl Trop Dis. 2012 Jan 31;6(7):e1758.
7. Hamilton J V, Klinkert M, Doenhoff MJ. Diagnosis of schistosomiasis: antibody detection, with notes on parasitological and antigen detection methods. Parasitology. 1998 Jan;117 Suppl:S41-57.
8. Doenhoff MJ, Butterworth AE, Hayes RJ, Sturrock RF, Ouma JH, Koech D, et al. Seroepidemiology and serodiagnosis of schistosomiasis in Kenya using crude and purified egg antigens of *Schistosoma mansoni* in ELISA. Trans R Soc Trop Med Hyg. 1993 Jan;87(1):42–8.
9. Pontes LA, Dias-Neto E, Rabello A. Detection by polymerase chain reaction of *Schistosoma mansoni* DNA in human serum and feces. Am J Trop Med Hyg. 2002 Feb;66(2):157–62.
10. King CH, Bertsch D. Meta-analysis of urine heme dipstick diagnosis of *Schistosoma haematobium* infection, including low-prevalence and previously treated populations. PLoS Neglected Tropical Diseases.2013; 7: e2431. doi: 10.1371/journal.pntd.0002431.
11. van Lieshout L, Roestenberg M. Clinical consequences of new diagnostic tools for intestinal parasites. Clin Microbiol Infect. 2015 Jun;21(6):520–8.
12. Utzinger J, Becker SL, van Lieshout L, van Dam GJ, Knopp S. New diagnostic tools in schistosomiasis. Clin Microbiol Infect. 2015 Jun;21(6):529–42.
13. WHO. Prevention and control of schistosomiasis and soil-transmitted helminthiasis. Geneva: WHO; 2002. World Health Organ Tech Rep Ser. No.: 912.
14. Hamburger J, Turetski T, Kapeller I, Deresiewicz R. Highly repeated short DNA sequences in the genome of *Schistosoma mansoni* recognized by a species-specific probe. Mol Biochem Parasitol. 1991 Jan;44(1):73–80.
15. Enk MJ, Lima AC, Drummond SC, Schall VT, Coelho PM. The effect of the number of stool samples on the observed prevalence and the infection intensity with *Schistosoma mansoni* among a population in an area of low transmission. Acta Trop. 2008 Jan;108(2–3):222–8.
16. Joseph L, Gyorkos TW, Bayesian CL. Estimation of disease prevalence and the parameters of diagnostic tests in the absence of a gold standard. Am J Epidemiol. 1995 Feb 1;141(3):263–72.
17. Simundic A-M. Measures of diagnostic accuracy basic definitions. International Federation of Clinical Chemistry and Laboratory Medicine (IFCC). 2008. [cited 2016 Jan 25]. Available from: http://www.ifcc.org/ifcc-communications-publications-division-(cpd)/ifcc public ations /ejifcc-(journal)/e-journal-volumes/ejifcc-2008-vol-19/vol-19-no-4/measures-of-diagnostic-accuracy-bas ic - definitions/
18. Pontes LA, Oliveira MC, Katz N, Dias-Neto E, Rabello A. Comparison of a polymerase chain reaction and the Kato-Katz technique for diagnosing infection with *Schistosoma mansoni.* Am J Trop Med Hyg. 2003 Jun;68(6):652–6.
19. Haggag SH, Abdullah SM. Molecular diagnosis of *Schistosoma mansoni* infection in human serum and feces by using Polymerase Chain Reaction. Life Sci J. 2011;8(2):398–404.
20. Sadek M, El Missiry A, El-Tonsy M, Hussein H, El Asmar M, Ebrahim S. Detection of *S. mansoni* DNA in human blood as a diagnostic method for schistosomiasis. Parasitol United J. 2008;1:31–6.
21. Hussein HM, El-Tonsy MM, Tawfik RA, Ahmed SA-E-G. Experimental study for early diagnosis of prepatent schistosomiasis mansoni by detection of free circulating DNA in serum. Parasitol Res. 2012 Jul;111(1):475–8.
22. Wichmann D, Poppert S, Von Thien H, Clerinx J, Dieckmann S, Jensenius M, et al. Prospective European-wide multicentre study on a blood based real-time PCR for the diagnosis of acute schistosomiasis. BMC Infect Dis. 2013;13:55.
23. Enk MJ, Oliveira E, Silva G, Rodrigues NB. Diagnostic accuracy and applicability of a PCR system for the detection of *Schistosoma mansoni* DNA in human urine samples from an endemic area. PLoS One. 2012 Jan;7(6):e38947.