Original Research Article

COMPARISON OF MICROSCOPY, BOECK AND DRBOLAV'S STOOL CULTURE MEDIUM AND BICHRO-LATEX ANTIBODY TEST WITH A REFERENCE ELISA ANTIGEN TEST. FOR *E.HISTOLYTICA* DIAGNOSIS IN CALABAR,NIGERIA

ABSTRACT

Objective: A definitive diagnosis of *E. histolytica* is important in the treatment of amoebiasis and to avoid unnecessary costs. This study's aim is to make a comparison of the different diagnostic method in the patients specimens defined as *E. histolytica/E.dispar* infection.

Materials And Methods: Faecal and serum specimens of 200 patients defined as symptomatic(diarrhea and dysentery) and asymptomatic (a case history of *E.histolytica* infection) was used for the study .Stool specimen was examined with microscopy (wet mount examination with 0.85% saline and Lugol's iodine and concentration technique), cultured in Boeck and drbolav's medium and anti-*E. histolytica* antibodies were investigated using a latex slide test. Stool samples were also examined by immunoassay methods for specific adhesin antigens (Wampole ® *E. histolytica* II antigen testing) which is the reference standard for comparison.

Result: The number of positive *E. histolytica* parasite in 200 samples were 12(6.0%) in microscopy,34(17%) in antibody test and 6(3.0%) in Boeck and drbolav's medium.The three test methods showed significant detection of *E.histolytica parasite*($p<0.05$).Microscropic method detected 100% of *E.histolytica* infection in symptomatic patients andBoeck and Drbohlav's culture medium detected 33.3%. However,the method of diagnosis is not associated with the detection *of E.histolytica* infection in asymptomatic and symptomatic Patients($p>0.05$).The diagnostic accuracy of the microscopy diagnostic method showed that sensitivity was 40.2%, specificity was 82.3%, PPV 39.6% and NPV 70.4% .The sensitivity was 86.6%, specificity was 70.6% PPV 87.6% and NPV 75.6% for bichro-latex antibody assay The sensitivity was 20.6 %, specificity was 50.6 %, PPV 34.6%, NPV 61.2% for Boeck and Drbohlav's culture medium

Conclusion: *E. histolytica* in stools by direct wet-smear microscopy and concentration technique can cause significant false positive results. To obtain a reliable diagnosis for *E. histolytica* and to avoid unnecessary treatment for this parasite,bichro-latex antibody assay is recommended because of its high specificity,sensitivity,positive predictive value and negative predictive value.

Keywords*: Entamoeba histolytica/E. dispar*, Amoebiasis, Entamoeba antigens, ELISA, antibody

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INTRODUCTION

Amoebiasis is parasitic disease caused by *Entamoeba histolytica*, The World Health Organization reported that *E. histolytica* causes approximately 50 million cases and 100,000 deaths annually [1-3]. The major cause of morbidity and mortalityin tropical African countries[2]. The majority of these infections are domiciled in the developing countries such as Nigeria [4]. Approximately 90% of infected individuals are asymptomatic carriers; the other 10% show clinical symptoms such as colitis, dysentery and extra-intestinal amoebiasis[3]. Clinical manifestation of extra-intestinal infection is amoebic liver abscess and a delay in diagnosis and treatment may cause fatality (4). Detection of the *E. histolytica* and its differentiation from the non-pathogenic *E.dispar* plays a major role in clinical management of the Patient [5]Laboratory diagnosis of intestinal amoebiasis in developing countries relies on labour-intensive method involving staining of stool sample and microscopy. The stool microscopy is routinely used in diagnosis of *E. histolytica* infection is unable to differentiate between *E histolytica*and the non-pathogenic amoeba *E dispar*[6-7], Laboratory diagnostic methods for amoebiasis are based on parasitological, immunological and molecular techniques [8]. The parasitological diagnosis is based on detection of cyst or trophozoites of *E. histolytica* in stool by microscopic examination. This technique is still practiced in many parasitology diagnostic laboratories, particularly in developing countries[3]. However , the limitation includes; the morphological similar nonpathogenic strain *E. dispar*, misdiagnosisand and over-treatment were common. The morphologies of *E. histolytica, E. dispar and E. moshkovskii* under the microscope are indistinguishable, although the presence of ingested red blood cells most likely indicates infection with *E. histolytica*. Moreover, although these three species can be differentiated morphologically from the other common amoebas, it is still a challenge for an inexperienced microscopist. Thus, the diagnostic sensitivity and specificity of microscopic examination to detect *E. histolytica* in stool is considered low[9-13].

Amoebiasis can also be diagnosed by antibody detection but majority of patients with intestinal amoebiasis have been exposed to *Entamoeba histolytica*, and developed IgG antibodies to this parasite which may persist for some time. Thus, definitive diagnosis using the available IgG antibody detection assays is a challenge because of the difficulty in differentiating past and current infections[14].

Stool culture followed by isoenzyme analysis was commonly used as a gold standard method to differentiate between *E. histolytica* and *E. dispar* . From the cultured amoeba, isoenzyme analysis is performedusing zymodeme enzymes as markers to identify the parasite[16]. However, isoenzyme analysis requires the use of cultured amoeba trophozoites which is tedious and time consuming[17-19]. 4 to 10 days are needed to grow the trophozoitesto a significant amount prior to performing starch-gelelectrophoresis, and the culture may not be always successful[20]. In reference laboratories, the success rate of establishing *E. histolytica* culture was reported to be between 50 and 70% [15]. The isoenzyme analysis of *E. histolytica* culture from clinical samples often gives false-negative result. There were also many samples that were positive by microscopy but were culture-negative[21].

In addition, a major problem that may arise during *E. histolytica* culture is the overgrowth of bacteria, other protozoan or fungi. Therefore, due to its low sensitivity, culture in combination with isoenzyme analysis, is not routinely used in diagnosis[22]. The disadvantages of the traditional parasitological techniques such as Stool microscopy, antibody test and stool culture

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have led to the current use of ELISAs for laboratory diagnosis of intestinal amoebiasis[8], ELISAs are usefulfor clinical and epidemiological studies, especially where molecularassays are not practical or available(19). The immunoassayis relatively simple and rapid, and canbe performed in most laboratories with minimum skills Tech Lab *E. histolytica* II ELISA is the most commonly used antigen detection test. It is the first generation kit in ELISA format produced in 1993 to specifically detect *E. histolytica* Gal/GalNAclectin in stool samples[19,21].This lectin protein is highly immunogenic and conserved and can be used to specifically detect *E. histolytica* due to the antigenic differences between the lectins of *E. histolytica* and *E. dispar*. This test showed an excellent correlation with nested PCR when tested with stool samples from people with diarrhea[21]. Moreover, this test was reported to have higher sensitivity (80 to 94%) and specificity (94 to 100%), as compared to both microscopy and culture[23,24]. Due to some limitations observed in the first generation TechLab ELISA kit, a second version of the kit called Tech Lab E. histolytica II was produced. In a study performed, It also demonstrated good levels of sensitivity (71 to 79%) and specificity (96 to 100%)when compared to real-time PCR for the diagnosis of *E. histolytica*[25,26]. Although, molecular detection techniques are highly sensitive and specific however, cost is still a barrier for their use as a routine laboratory test method and research in most endemic area [22]. The aim of these study is to compare different diagnostic method in diagnosis of amoebiasis with a reference Tech Lab *E. histolytica* II ELISA with high specificity for *E. histolytica* parasite These will help in recommendation for highly sensitive and specific tests that are rapid and cost-effective for use in developing countries such as Nigeria where the disease is endemic

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MATERIALS AND METHOD

Study Design

A cross sectional study carried out in general hospital in Calabar,Cross River state from patients diagnosed of asymptomatic and symptomatic dysenteric patients from January –December, 2013

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Ethical Approval

Ethical approval was received for this study from the ethical committee from cross river state ministry of health. Informed consent was obtained from each patient.

Enrollment criteria

Stool and blood specimen was collected from patients presenting to the general hospital calabar with acute and persistent dysentery for symptomatic patients and no clinical manifestation of amoebiasis but a history of the infection for asymptomatic patients within the 12 months period of study were enlisted having consented to participate and fulfilled the inclusion criteria which included acute or persistent diarrhoea and dysenteric syndrome for symptomatic and no clinical manifestation but a history of the infection.

Patients with diarrhoea or dysentery on antimicrobial agents were excluded.Patients visiting the hospital for reasons other than diarrhoea and had no diarrhoeal illness within the last 2 weekswere used as control.

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Specimen collection and processing

This study was carried out with stool and serum samples. Serumsamples were obtained from aseptically collected blood from200patients clinically diagnosed of asymptomatic and symptomatic diarrhoea or dysentery. One pie size of stool sample from each of the patient was immediately examined using microscopy and formol ether concentration technique. The remaining stool specimens were stored at −20°C until needed for ELISA antigen tests. For *E. histolytica* antibody assays,the serum was separated by centrifugation of the blood at 3,000 r.p.m for 10 minutes at room temperature to obtain the serum. 3–4 mL of the patients' serum sample were collected and stored at −20°C until required for use.

Microscopy

Clinically diagnosed dysenteric and diarrheic specimens from infections or a history of the infection from general hospital calabar were examined by directs smear method according to the method reported by Cheesbrough (2005)[27].A loopful of saline is placed on one end of a slide and another drop iodine on the other end.Using a wire loop a small amount of the faeces is mixed with the normal saline and iodine on the slide and covered with cover slip, then examined systermatically with the low and high power (×10) and (×40) objectives for trophozoites of *E.histolytica* parasite.of

Formol- Ether Concentration Method

The stool samples were analysed using the Formol-Ether concentration method of Cheesbrough (2005) [27]. The emulsified faecal samples were filtered in two-layered gauze and the filtrate transferred to a conical centrifuge tube containing equal volume of ether and centrifuged for 1 minute at 3,000rpm. After discarding the faecal debris and ether, the sediment was transferred to a clean glass slide and a drop of iodine was added. The entire preparation covered with a cover slip and examined microscopically under x40 objective to identify the *E.histolytica* trophozoites

Bichro-Latex AntibodyAmibe Fumouze Test (Fumouze Diagnostics, Levallois-Perret, France)

To search for *E. histolytica* antibodies in the serum, 20 µL of serum from each test were transferred into sterile Eppendorf tubes. The serum specimens were diluted with two drops of diluent in the kit. Then a drop of reagent and a drop of diluted patient serum were added on the test slide, and the mixture was rotated in a rotator for 5 min. Finally, agglutination observed specimens were evaluated as positive. Positive and Negative control were included in each test batch for accurate diagnosis.

The Boeck and Drbohlav's Stool culture medium

The Boeck and Drbohlav's medium was used to culture the dysentric and diarrhoeic stool with some modifications as described by Sawangjaroen et al, (1993). Calf serum (10%) was used as a substitute of horse serum and bijoux bottle were used as parasite culture tube. Just before culture, a drop of sterilized rice starch (1mg) was included to the medium. Then a small amount of faeces wereinoculated in the culture medium and incubated at 37^{O}C for 48 hours. After 48 hours incubation, the culture fluid in the tube was mixed and then observed on a microscope for amoebic growth, the culture was incubated at 37^{o}C and *Entoamoeba histolytica* trophozoites along with related bacteria were sub cultured at 48 hours intervals

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ELISA Wampole *E. histolytica* II Test (Techlab.)

This test is a monoclonal ELISA test that rapidly detects the adhesins of E. histolytica (specific antigen) in stools. The monoclonal antibody-peroxidase conjugate used in the test was the specific adhesin for *E. histolytica*. Frozen stool samples were dissolved at room temperature before starting the test, and test procedures were performed according to the instructions in the test kit.

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Reference Standard

A reference standard for a positive result was defined as a "Positive" result when *E. histolytica* was detected by antigen testing. Theyare ELISA Wampole*E. Histolytica* II Test (Techlab.), referrence standard for a negative result was defined as a "negative" result by ELISA Wampole *E. Histolytica* II Test (Techlab.)

Statistical Analysis

Correlation of the diagnostic parameters of Microscopy, Bichro-latex antibody and culure with ELISA antigentest for diagnosis of *E.histolytica* test as a gold standard was done usingchi-square and kappa's test [17].

RESULT

TABLE 1 Comparison of different methods for diagnosi of E. histolytica

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Method	NSE	NP	PP(%)
Microscopy	**200**	**12**	**06**
Antibody	**200**	**34**	**17**
Culture	**200**	**06**	**03**

NSE =Number of samples examined NP= number positive PP= percentage positive X^2=27.456, df=2, p=0.01

Table 2 comparison of different diagnostic methods in diagnosis of *E.histolytica* infection in symptomatic and asymptomatic patients

Method	NPS	NAS(%)	NSS(%)
Microscopy	**12**	0 (0.00%)	12(100)
Antibody	**34**	6(17.6)	28(82.4)
Culture	**6**	2(33.3)	4(66.66)

NPS= number positive NAS = Nunmber of asymptomatic samples NSS= number of symptomatic samples X^2=3.8,df=2,p=0.150

Table 3 Diagnostic accuracy of different diagnostic methods for *E. histolytica*

Method	Sensitivity (%)	Spcificity(%)	PPV(%)	NPV(%)
Microscopy	40.2	82.3	39.6	70.4
Antibody	86.6	70.6	87.6	75.6
Culture	20.6	50.6	34.6	61.2

PPV= PositivePredictiveValue NPV = PositivePredictiveValue

The comparison of diagnosis method for diagnosis of *E.histolytica* parasite *is* shown in Table 1. On the basis of comparison diagnostic method, 6.0% tested positive to the microcopy method,17.0% tested positive for bichro-latex antibody assay and 3% were positive by Boeck and Drbohlav's culture medium. There was significant difference in the diagnostic method for *E.histolytica* parasite (Table 1).The 3 test methods showed significant detection of *E.histolytica parasite.* Both chi square andKappa's test analysis showed that the diagnostic methods significantly detected *E.histolytica* parasite (p=0.001).

Microscopic method detected 100% of *E.histolytica* infection in symptomatic patients ,82.4% was detected in Bichro-latex antibody assay for symptomatic patients and 17.6% in asymptomatic subjects and 66.66% in symptomatic patients and 33.3% for asymptomatic subject in Boeck and Drbohlav's culture medium(Table 2).However,the method of diagnosis is not associated with the detection of *E.histolytica* infection in asymptomatic and symptomatic Patient (p=0.150).

The diagnostic accuracy of the microscopy diagnostic method showed that sensitivity was 40.2%, specificity was 82.3%,positive predictive value PPV 39.6% and negative predictive value 70.4%.

The sensitivity was 86.6%, specificity was 70.6% ,positive predictive value 87.6% and negative predictive value of 75.6% for bichro-latex antibody assay The sensitivity was 20.6 %,

specificity was 50.6 %, positive predictive value 34.6%, negative predictive value 61.2% for Boeck and Drbohlav's culture medium.(Table 3)

DISCUSSION

Amoebiasis, an enteric protozoan disease caused by *Entamoeba histolytica*, is a public health problem in many developing countries [28]. Detection of the pathogenic *E. histolytica* and its differentiation from the non-pathogenic *Entamoeba sp*. is very important in the clinical management of patients[28]. Laboratory diagnosis of intestinal amoebiasis in developing countries relies on labour-intensive and insensitive methods involving staining of stool sample and microscopy. The presence of ingested RBCs in the cytoplasm of the trophozoites is commonly regarded as diagnostic of *E. histolytica* infection. However, Haque *et al*, 1998 found that 16% of *E. dispar* isolates had ingested RBCs; thus, this distinction between the two species is not absolute [21].

In the present study, different diagnostic methods such as microscopy,antibody and culture was standardized against ELISA antigen techniques for the detection of *E.histolytica* parasite. The detection of *E.histolytica* was 6%, 17% and 3% from microscopy,antibody and Culture diagnostic methods, respectively. This is in contrast with Ozer *et al*.,2011 [31] detected *E. histolytica/dispar* cysts and/or trophozoites in 2.2% of stool samples by direct examination using the saline-iodine method and detected 0.7% in samples using the ELISA method. Gözkenç *etal*.2007 [32] detected in 1.3% using saline-iodine preparation methods after sedimentation. Tuncay *et al*.2007[33] investigated the stool samples of patients using Microscopic preparation from iodine and saline, They reported very low *E.histolytica* parasite detection compared to other test and emphasized the necessity of working with specific ELISA for *E. histolytica* detection. Zeyrek *et al*.,2006 [34]detected specific *E. histolytica* antigen positivity in 21.7% of cases using ELISA and microscopy positivity in 26.4%,which is higher than *E.histolytica* reported from the same diagnostic method in this study, the difference is attributed to study population and environmental factors. However, Tüzemen and Dogan ,2014 (35) detected positivity in 54.7% of the samples by seeing suspected amoeba cysts/trophozoites using direct microscopy, 15.5% using ELISA and in 7.1% using culture.These results is higher than previously reported ,the Increased detection of *E.histolytica* parasite is due to the large sample size as our study was limited to only 200 sample for the three diagnostic methods. These two researchers reported that the prevalence of *E. histolytica/E. dispar* ranged from 0.2–45.9%. In different regions between the years 2008–2013, they suggested using combined methods and evaluating them together with the clinical findings in the laboratory diagnosis of patients with amoebiasis.There was significant difference in the three diagnostic methods for detection of *E.histolytica* parasite indicating that the three methods can be used in diagnosis of the parasite.

The antigen test of Yuksel *et al*.2011 [36]found that 7% of the stool samples of the patients with clinical gastroenteritis symptoms were positive for *E. histolytica/E. dispar*. They also reported that, due to the low sensitivity of direct microscopy, the use of antigen detection methods by ELISA would be appropriate to confirm diagnosis in patients with suspected amoebiasis. Aydin *et al*.,2012[37] stated that the preferred method is permanent trichrome staining because it allows faeces to be examined later for the identification of the internal structure of the protozoa.

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Perreira*et al*. 2014[38] , found goo sensitivity and 100% specificity in stool antigen testing by ELISA for the diagnosis of amoebiasis, and they suggested the use of this method as a diagnostic test. Kraoul *et al*.,1997[39] compared the IHA, latex agglutination and the ELISA test. Sensitivity and specificity of the tests were found as 97.6% and 97% for IHA, 90.7% and 95% for latex agglutination and 93% and 100% for ELISA, respectively. Singh *et al*,2009 [40] divided stool samples into direct microscopy to detect the trophozoites and erythrocytes and trichrome and/or lugol staining to detect cysts and trophozoites. Singh *et al.* 2007[40] expressed that the presence of trophozoites in red blood cells differentiates *E. histolytica* from *E. dispar*. Tanyüksel and Petri ,2005 [30] reported that sensitivity and specificity were about 60% positive and between 10–50% for microscopy, 95% for an ELISA test based on the antigen in the stool and between 90%–85% for the ELISA test based on the antibody in serum. Goñi *et al* ,2009[41] argued that microscopy and PCR are the gold standard reference techniques. In their studies that took microscopy as the gold standard, they found 17.1% and 96.6% for antigen testing sensitivity and specificity and 24.4% and 97.5% for ELISA, respectively. Tüzemen and Dogan,2014 [35] took multiplex PCR for a reference, and they found sensitivity and specificity at 66.7% and 77.4% for direct microscopy, 44.4% and 83.5% for trichrome staining and 11.1% and 91.3% for ELISA, respectively there was no a significant difference between the three diagnostic methods indicating thateither of both methods can be used in diagnosing the parasite.

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The results from this study detected 12(100%),28(82.4%),and 4(66.7%) in symptomatic patients in microscopy, antibody and culture diagnostic methods which is higher than the *E.histolytica* parasites detected in asymptomatic subjects. However,*E.histolytica* parasite was not detected in microcopy diagnostic method these is explained that trophozoite /parasite presents in stool there must be active infection [42].Although,Infection with *E. histolytica*/*E. dispar* can result in differentclinical presentations: asymptomatic infection, symptomaticinfection without tissue invasion, and symptomaticinfection with tissue invasion. The majority of infectionswith *E. histolytica*/*E. dispar* are asymptomatic. Individualswith such infections will have a negative or weak serologic response[42] this accounts for decrease in dectection of *E.histolytica* parasite in antibody diagnostic method. The study also revealed that the three diagnostic methods was not significantly associated with the detection of *E.histolytica* parasite in asymptomatic and symptomatic patients.The implication from this results is that the three methods can only detects the parasite in symptomatic patients significantly.

The sensitivity of 86.6 % the antibody diagnostic method was high ,when compared to the two other diagnostic method .This is in line with Kraoul *et al*,[24] who reported 90.7 latex agglutination for antibody and 93.0 % for ELISA antibody although slightly higher than the result. The difference is as a results diagnostic method.The specificity was higher 82.3% in microscopy diagnostic method;this is in contrast to other studies Singh *et al*.,2009 Tanyüksel and Petri,2005. Goñi *etal.* 2012 [41]. Tüzemen and Dogan,2014 [35] which reported lower specificity. This is explained by the stool concentration technique for microscopy in this study. The positive predictive value and negative predictive value 87.6% and 75.6% respectively was highest for the antibody diagnostic method. The results suggest that antibody diagnostic method showed good performance in detecting *E.histolytica* parasite.

The clinical implications of this study are significant since. Therefore, most patients identified with *E. histolytica/E. dispar* complex infection by microscopy in Calabar received unnecessary therapy. Use of simple cost effective latex agglutination antibody test would allowed for a specific diagnosis and remove the need for unnecessary chemotherapy with its attendant costs, risk of side effects, danger of drug resistance, and potential mistreatment of another disease. Although previous studies suggest that the rate of false-positive results for serology is higher [21] serological based antibody test may help identify *E. histolytica*–infected patients

CONCLUSION

In conclusion, we recommend that latex agglutination should be considered for used in routine laboratory screening test and epidemiological studies in areas where amoebiasis is endemic and where facilities with ELISA and PCR are not available considering its speed, simplicity and low cost together with it's good to moderate accuracy and specificity.

CONSENT According to laid down international standards written informed consent was obtained from the patient (or other approved parties) for publication of this study. Written informed consent was obtained from all study participants

ETHICAL APPROVAL Ethical approval was sought and obtained from the appropriate ethics committee. All tests were performed in accordance with laid down standards. Ethical clearance was sought and obtained from the ethical committees of the University of Calabar Teaching Hospital.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

References

1). Stanley SL, (2003) Amoebiasis. *Lancet* 361:1025–1034

2). Ibrahim SS, El-Matarawy OM, Ghieth MA, Sarea EYA, El-BadryAA (2015) Copro-prevalence and estimated risk of *Entamoeba histolytica*in diarrheic patients at Beni- Suef, Egypt. *World Journal of Microbiology Biotechnology* 31:385–390C05–DC08

3). Al-AreeqiMA, Sady H, Al-MekhlafiHM, Anuar TS, Al-AdhroeyAH, Atroosh WM e(2017) First molecular epidemiology of*Entamoeba histolytica*, *E. dispar*and *E. moshkovskii*infections inYemen: different species-specific associated riskfactors. *Tropical Med Int Health* 22:493–504

4)Stauffer W.Abd-Alla,.M.Ravdin J.I,(2006)Prevalence and incidence of *E.histolytica* in South Africa and Egypt. *Arch.Med. Res.*7:266-299

5) Ravdin JI, Stauffer M M, (2005). *Entamoeba histolytica* (Amoebiasis). In Mendell, G. L., Benneth, J. E, Dolin,R. (ed) Mendell, Doglas and Benneth) Principles and Practice of Infectious Diseases. 6th ed. *Philadelphia, P. A.*
Churchill Livingstone

6) Parija SC,Mandal J, Ponnambath DK, (2014) Laboratory methods of identification of *Entamoeba histolytica*and its differentiationfrom look-alike *Entamoeba spp*. *Trop Parasitol* 4:90

7)Shakir JM ,(2015) Evaluation of multiplex real-time PCR for detection
of three diarrhea causing intestinal protozoa. *Med Sci* 5:783–786

8)Saidin S,Othman N, Noordin R,(2019) Update of laboratory diagnosis of Amoebiasis.,*European Journal of clinical Microbiology and infectious disease* 38:15-38

9). Haque R, Neville LM, Hahn P, PetriWA (1995) Rapid diagnosis of*Entamoeba* infection by using *Entamoeba dispar*and *Entamoebahistolytica* stool antigen detection kits. *J Clin Microbiol* 33:2558–2561

10).World Health Organization (WHO) (1997) UNESCO report of aconsultation of experts on amoebiasis. *Wkly Epidemiol Rec* 72:97–99

11). Tanyuksel M, PetriWA, (2003) Laboratory diagnosis of amebiasis.*Clin Microbiol Rev* 16:713–729

12)Verkerke HP, Hanbury B, Siddique A, Samie A, Haque R, HerbeinJ, Petri WA (2015) Multisite clinical evaluation of a rapid test for *Entamoeba histolytica i*n stool. *J Clin Microbiol* 53:493–497

13)Morshed M, Cherian SS, Lo T, LeeMK,Wong Q, Hoang L, (2017)Superiority of PCR againstmicroscopy for diagnosing *Entamoebahistolytica*in liver abscess samples. *Can J Infect Dis MedMicrobiol* 2:1–3
.
14). Aguayo-Patrón S, Castillo-Fimbres R, Quihui-Cota L, de la BarcaAMC ,(2017) Use of real-time polymerase chain reaction identify *Entamoeba histolytica* in schoolchildren from northwestMexico. *J Infect Dev Countries* 11:800–805

15). Clark CG, Diamond LS, (2002) Methods for cultivation ofluminal parasitic protest of clinical importance. *Clin Microbiol Rev* 15:329–341

16). Dhanalakshmi S, Parija SC, (2016) Seroprevalence of*Entamoeba histolytica*from a tertiary care hospital, SouthIndia. *Trop Parasitol* 6:78

17). Alireza B, Mostafa H, Ahmed N, GehadEA,.(20150 Simple definition and calculation ofaccuracy, sensitivity and specificity. *Emergency stat*. 3(2):48-49

18)Ujang JA, Kwan SH, Ismail MN, Lim BH, Noordin R, Othman N ,(2016) Proteome analysis of excretory-secretoryof *Entamoeba histolytica*HM1: IMSS via LC–ESI–MS/MS andLC–MALDI–TOF/TOF. *Clin Proteomic*13:3

19). Haque R, Faruque ASG, Hahn P, Lyerly DM, Petri WA, (1997)*Entamoebahistolytica* and *Entamoeba dispar* infection in childrenin Bangladesh. *J Infec Dis* 175:734–736

20). Haque R, PetriWA ,(2006) Diagnosis of amoebiasis in Bangladesh.*Arch MedRes* 37:272–275

21). Uslu H, Aktas O, Uyanik MH, (2016) Comparison of variousmethods in the diagnosis of *Entamoeba histolytica*in stool andserum specimens. *Eur J Med* 48:124

22). Verkerke HP, Hanbury B, Siddique A, Samie A, Haque R, HerbeinJ, Petri WA (2015) Multisite clinical evaluation of a rapid test for*Entamoeba histolytica*in stool. *J Clin Microbiol* 53:493–49

23). Haque R, Neville LM, Hahn P, PetriWA, (1995) Rapid diagnosis ofEntamoeba infection byusing Entamoeba and *Entamoebahistolytica* stool antigen detection kits. *J Clin Microbiol* 33:2558–256125.

24) Haque R,Kress K,Wood S, Jackson TF, LyerlyD,Wilkins T, PetriWA (1993) Diagnosis of pathogenic Entamoeba histolytica infectionusing a stool ELISA based on monoclonal antibodies to thegalactose-specific adhesin. *J Infect Dis* 167:247–249

25). Roy S, Kabir M, Mondal D, Ali IKM, PetriWA, Haque R (2005)Real-time-PCR assay for diagnosis of *Entamoeba histolytica* infection.*J Clin Microbiol* 43:2168–2172

26). Visser LG, Verweij JJ, Van Esbroeck M, Edeling WM, Clerinx J,Polderman AM (2006) Diagnostic methods for differentiation of *Entamoeba histolytica* and *Entamoeba dispar* in carriers: performance and clinical implications in a non-endemic setting. *Int JMed Microbiol* 296:397–403

27)Cheesbrough,M.(2005).District Laboratory practice inTropical Countries. *Cambridge University Press.*200-205

28) Ali IK, Clark CG, Petri WA, Jr (2008) Molecular epidemiology of amebiasis. *Infect Genet Evol*. 8:698707.http://dx.doi.org/10.1016/j.meegid.2008.05.004.

29)WHO/PAHO/UNESCO (1997)Report A consultation with experts on amoebiasis. Mexico City, Mexico, 28–29 January,. *Epidemiol Bull*. 1997;18:13–4.

30)Tanyuksel, M., W. A. Petri, Jr.**(**2003). Laboratorydiagnosis of amebiasis. *Clin. Microbiol. Rev*. **16:**713–729.

31) Özer TT, Yula E, Deveci Ö, Tekin A, Durmaz S, Yanık K.(2011) Investigation of *Entamoeba histolytica* in stool specimens by direct microscopic examination and ELISA in a hospital. *Dicle Med Journal* ;38:294–7. http://dx.doi.org/10.5798/diclemedj.0921.2011.03.0034.

32) Gözkenç N, Şahin İ, Yazar S.(2007) Amibiyazis tanısında nativ-lugol, sedimantasyon ve trikrom boyama yöntemlerinin karşılaştırılması Sağlık Bilimleri Derg. ;16:49–55.

33) Tuncay S, Inceboz T, Över L, (2007) The evaluation of the techniques used for diagnosis of *Entamoeba histolytica* in stool specimens. *Turkiye Parazitol Derg*. 31:188–93.

34)Zeyrek FY, Özbilge H, Yüksel MF, Zeyrek CD, Sirmatel F(2006) Parasitic fauna and the frequency of *Entamoeba histolytica/Entamoeba dispar* detected by ELISA in stool samples in Sanliurfa, Turkey .*Turkiye Parazitol Derg*. 30:95–8.

35). Tüzemen NÜ, Doğan N,(2014) Comparison of direct microscopy, culture, ELISA and molecular methods for diagnosis of *Entamoeba histolytica*. *Mikrobiyol Bul*. 48:114–22.

36). Yuksel P, Celik DG, Gungordu Z,(2014). Dişki Örneklerinde Elisa Yöntemiyle *Entamoeba Histolytica* Lektin Antijeninin Gösterilmesi: Üç Yıllık Veriler. *KlimikDerg*.;24:150–3. http://dx.doi.org/10.5152/kd.2011.37.

37). Aydın M, Adıyaman G, Kaya T, Kuştimur S, Al FD.(2012). Comparison of Conventional and Commercial Trichrome Staining Methods for Detecting Protozoan in Stool Samples. *Kafkas Univ Vet Fak Derg*.18(Suppl-A):A155–A159.

38).Pereira VV, Conceição A da S, Maximiano LHS, Belligoli L de QG, Silva ES da, Pereira VV, (2014) Laboratory diagnosis of amebiasis in asample of students from southeastern Brazil and a comparison of microscopy with enzyme-linked immunosorbent assay for screening of infectionswith Entamoeba sp. *Revista da SociedadeBrasileira de Medicina Tropical*.;47(1):52–6.

39). Kraoul L, Adjmi H, Lavarde V, Pays JF, Tourte-Schaefer C, Hennequin C. (1997)Evaluation of a rapid enzyme immunoassay for diagnosis of hepatic amoebiasis. *J Clin Microbiol*. ;35:1530–2.

40). Singh A, Houpt E, Petri WA.(2009) Rapid Diagnosis of Intestinal Parasitic Protozoa, with a Focus on *Entamoeba histolytica*. *Interdiscip Perspect Infect Dis*.;2009:547090. http://dx.doi.org/10.1155/2009/547090

41). Goñi P, Martín B, Villacampa M, (2012). Evaluation of an immunochromatographic dip strip test for simultaneous detection of *Cryptosporidium spp*, *Giardia duodenalis*, and *Entamoeba histolytica* antigens in human faecal samples. Eur J Clin Microbiol Infect Dis. 2012;31:2077–82. http://dx.doi.org/10.1007/s10096-012-1544-7

42). **World Health Organization.** 1997. Amoebiasis. *Wkly.Epidemiol. Rec.* **72:**97–100.
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