European Journal of Clinical Microbiology & Infectious Diseases (2019) 38:15–38
https://doi.org/10.1007/s10096-018-3379-3

REVIEW

Update on laboratory diagnosis of amoebiasis

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Received: 12 July 2018 / Accepted: 7 September 2018 / Published online: 25 September 2018

Abstract

Amoebiasis, an enteric protozoan disease caused by *Entamoeba histolytica*, is a public health problem in many developing countries, causing up to 100,000 fatal cases annually. Detection of the pathogenic *E. histolytica* and its differentiation from the non-pathogenic *Entamoeba* spp. play a crucial role in the clinical management of patients. Laboratory diagnosis of intestinal amoebiasis in developing countries still relies on labour-intensive and insensitive methods involving staining of stool sample and microscopy. Newer and more sensitive methods include a variety of antigen detection ELISAs and rapid tests; however, their diagnostic sensitivity and specificity seem to vary between studies, and some tests do not distinguish among the *Entamoeba* species. Molecular detection techniques are highly sensitive and specific and isothermal amplification approaches may be developed into field-applicable tests; however, cost is still a barrier for their use as a routine laboratory test method in most endemic areas. Laboratory diagnosis of extraintestinal amoebiasis faces challenges of lack of definitive detection of current infection and commercially available point-of-care tests. For both types of amoebiasis, there is still a need for highly sensitive and specific tests that are rapid and cost-effective for use in developing countries where the disease is prevalent. In recent years, new molecules of diagnostic value are being discovered and new tests developed. The advances in 'omics' technologies are enabling discoveries of new biomarkers that may help distinguish between different infection stages.

Keywords Amoebiasis · *Entamoeba histolytica* · *Entamoeba dispar* · Laboratory · Diagnosis

Introduction

Amoebiasis is still a big challenge to public health in many regions, especially in the 'bottom billion' countries where poverty and low income is prevalent, and complex challenges are hindering their economic development. Areas with high rates of amoebic infection include parts of India, Bangladesh, tropical African countries, Brazil and Mexico, China, and South-east Asia [1, 2]. It is estimated to affect 50 million people worldwide and causes up to 100,000 deaths annually [1, 3]. Approximately 90% of infected individuals are asymptomatic carriers; the other 10% show clinical symptoms such as colitis, dysentery and extraintestinal amoebiasis [3]. The most common clinical manifestation of extraintestinal infection is amoebic liver abscess (ALA) and a delay in diagnosis and treatment may cause fatality [4]. Despite the prevalence of amoebiasis, there is still no vaccine to prevent this disease [5]. Human infection is usually found in areas with poor sanitary conditions, inadequate water treatment and low socio-economic status. The only reservoir is human, and infection occurs via food, water or hands contaminated with cyst-containing fecal material. Human to human transmission has been reported through oral-genital and oral-anal contact, especially among homosexuals [6] and those with poor personal hygiene [7].

Diagnosis of intestinal amoebiasis relies on clinical symptoms and laboratory test results. Continuous improvement of health programmes, as well as monitoring and mapping the prevalence of amoebiasis is needed and this requires good diagnostic tools. This review describes the laboratory diagnosis of amoebiasis. Other than the conventional methods, a substantial amount of work has been carried out to develop new and improved serological and molecular diagnostic tests for both clinical and research purposes.

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Laboratory diagnosis of amoebiasis

Laboratory diagnostic methods for amoebiasis are based on parasitological, immunological and molecular techniques. The microscopic observation of the parasite in stool, body fluid or tissue sample is considered as the 'gold standard' in diagnosis. Patients in endemic areas with clinical signs and symptoms that include gastrointestinal discomfort and watery or bloody diarrhoea should be suspected of intestinal amoebiasis. The laboratory diagnosis of intestinal disease can be made by microscopy, culture, isoenzyme analysis, antigen detection test, molecular-based test and point-of-care (POC) test.

The laboratory diagnosis of extraintestinal amoebiasis is different from intestinal amoebiasis in two ways. First, most patients with extraintestinal amoebiasis, especially ALA, do not have concurrent amoebic colitis. Thus, analysis of stool sample is generally not performed for suspected ALA cases, unless intestinal symptoms are present as well. Second, 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 [8, 9].

Intestinal amoebiasis

Microscopic examination

The visual demonstration of *E. histolytica* cysts and/or trophozoites in stool or colonic mucosa of patients can be performed by microscopic examination. This technique is still frequently practiced in many parasitology diagnostic laboratories, particularly in developing countries [3]. The direct examination of a saline wet mount of fresh sample (with or without iodine as temporary stain) under a microscope is not a sensitive method [10]. To identify motile trophozoites (which may contain red blood cells), the stool samples need to be examined within 1 h of collection. Therefore, if the examination cannot be performed immediately, the stool sample should be preserved in polyvinyl alcohol (PVA), Schaudinn's fixative or sodium acetate-acetic acid-formalin (SAF) [11]. The possibility of observing trophozoites is higher in loose stools, which contain mucous, pus and trace amounts of occult blood, whereas cysts can be observed in both formed and loose stools [12]. Permanent stain of the stool smear should be examined to enable the morphology, size and number of the nuclei to be clearly observed. Stains such as methylene blue, Giemsa, Wright's and iodine-trichrome can be used for the staining, however for routine use, the modified iron haematoxylin and Wheatley's trichrome stains are recommended [3]. Even though microscopic examination allows visualization of the parasite and hence provides a definitive diagnosis, it has several limitations. Before the morphological similar non-pathogenic strain *E. dispar* was discovered, misdiagnosis 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 (*E. coli*, *E. hartmanni*, *E. polecki* and *I. butschlii*), it is still a challenge for an inexperienced technician. Thus, the diagnostic sensitivity and specificity of microscopic examination to detect *E. histolytica* in stool is considered low [13–17].

Biochemical methods: culture and isoenzyme analysis

Previously, stool culture followed by isoenzyme analysis was commonly used as a gold standard method to differentiate between *E. histolytica* and *E. dispar* [18]. Other than faecal sample, rectal biopsy or liver abscess aspirate can also be used to culture *E. histolytica*. Pus aspirate from liver of an ALA patient is normally sterile thus it is necessary to add a bacterium or a trypanosomatid before introducing the amoebae into a xenic culture [18–20].

From the cultured amoeba, isoenzyme analysis is performed using zymodeme enzymes as markers [21]. A zymodeme is a cluster of amoeba strains that has the same electrophoretic pattern and mobility for a few enzymes. Examples of the enzymes are hexokinase, decarboxylating malate dehydrogenase, glucose phosphate isomerase and phosphoglucomutase isoenzyme [22]. There are 24 different established zymodemes in which 21 are from human isolates (nine *E. histolytica* and 12 *E. dispar*) and another three zymodemes from experimentally cultured amoeba strains. Since *E. histolytica* and *E. dispar* have genetically different hexokinase enzymes, it is reliable in discriminating between the two species. Three zymodeme bands for *E. histolytica* (II, XIV, and XIX) as compared to only one band for *E. dispar* (I) can be used to differentiate the two *Entamoeba* species [23].

However, isoenzyme analysis requires the use of cultured amoeba trophozoites which is tedious and time consuming [12, 24–26]. Four to 10 days are needed to grow the trophozoites to a significant amount prior to performing starch-gel electrophoresis, and the culture may not be always successful [16]. In reference laboratories, the success rate of establishing *E. histolytica* culture was reported to be between 50 and 70% [18]. 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 [27]. In addition, a major problem that may arise during *E. histolytica* culture is the overgrowth of bacteria, other protozoan or fungi [18]. Therefore, due to its low sensitivity, culture in combination with isoenzyme analysis, is not routinely used in diagnosis [28]. This technique is more

suitable for research rather than as a primary diagnostic tool. Molecular diagnosis has now replaced isoenzyme analysis as the preferred method to identify *Entamoeba* species.

Antigen detection ELISA

The disadvantages of the traditional parasitological techniques have led to the current use of coproantigen ELISAs for laboratory diagnosis of intestinal amoebiasis. ELISAs are useful for clinical and epidemiological studies, especially where molecular assays are not practical or available [25, 26]. The immunoassay is relatively simple and rapid, and can be performed in most laboratories. TechLab *E. histolytica* II ELISA (TechLab, Blacksburg, VA, USA) and *Entamoeba* CELISA PATH kit (Cellabs, Brookvale, Australia) use monoclonal antibodies against *E. histolytica* Gal/GalNAc lectin. Other commercial ELISA kits include Optimum S kit (Merlin Diagnostika, Bernheim-Hersel, Germany) and ProSpecT ELISA (Remel Inc., Lenexa, KS, USA). The former detects serine-rich antigen of *E. histolytica* while the latter detects a specific antigen (EHSA) from *E. histolytica/E. dispar* [15]. Comparison of diagnostic sensitivity and specificity of these kits obtained from different studies are presented in Table 1. The most commonly used antigen detection test is the *E. histolytica* TechLab kit. It is the first generation kit in ELISA format produced in 1993 to specifically detect *E. histolytica* Gal/GalNAc lectin in stool samples [26, 27]. 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*. According to Haque et al. [27], this test showed an excellent correlation with nested PCR when tested with stool samples from people with diarrhoea. Moreover, this test was reported to have higher sensitivity (80 to 94%) and specificity (94 to 100%), as compared to both microscopy and culture [13, 25]. However, in a study by Gonin and Trudel [55], *E. histolytica* TechLab kit was found to show reduced diagnostic sensitivity and specificity compared to microscopy and PCR in discriminating between *E. histolytica* and *E. dispar*.

Due to some limitations observed in the first generation TechLab ELISA kit, a second version of the kit called TechLab *E. histolytica* II was produced. In a study performed in Bangladesh, it was reported to display higher sensitivity (86% to 95%) and specificity (93% to 100%) when compared to microscopy and culture [13, 28]. 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* [30, 31, 55]. In addition, Haque et al. [56] also used this kit on serum and liver abscess samples of patients. The result showed that, prior to treatment with metronidazole, 96% (22/23) and 100% (3/3) of the ALA patients can be detected to have the lectin antigen in their serum and liver abscess samples, respectively. However, after several days of the treatment, the diagnostic sensitivity decreased to 33% (32/98) and 41% (11/27) for serum and liver abscess, respectively. This is likely due to the reduction of lectin in the samples post-therapy. In contrast, another study which was performed in the village of Borbòn, Ecuador, reported that TechLab II *E. histolytica* showed low diagnostic sensitivity (14.3%) for detection of *E. histolytica* antigen when compared to culture and zymodeme analysis [29]. Visser et al. [31] also found that the kit lacked sensitivity for a reliable diagnosis of *E. histolytica* infection among carriers of the parasite in a non-endemic area. In other studies conducted in North India and Baghdad, Iraq, the sensitivity and specificity of this test was reported to be 20–60% and 86.7–93.4%, respectively, when compared with microscopy-positive *E. histolytica*, *E. dispar*, or *E. moshkovskii* [32, 33].

ProSpecT ELISA is a microplate immunoassay assay that detects both *E. histolytica* and *E. dispar* antigens. In comparison with conventional microscopy, the sensitivity and specificity of this test was reported to be 78% and 99%, respectively [35]. In another study by Gatti et al. [29], this assay was found to be 54.5% sensitive and 94% specific for detection of *E. histolytica/E. dispar* as compared to culture and zymodeme analysis. In Australia, Stark and colleagues [57] evaluated the use of the CELISA PATH and TechLab *E. histolytica* II kits to detect *E. histolytica* using PCR as the reference standard. Both kits use monoclonal antibody against the Gal/GalNAc lectin of *E. histolytica*. The former showed 28% sensitivity and 100% specificity, while the latter showed very low sensitivity and specificity. The TechLab *E. histolytica* II kit required 10,000 trophozoites/well for positive result, hence less sensitive as compared to *Entamoeba* CELISA PATH kit, which required approximately 1 000 trophozoites/well. It was suggested that the different amounts of antibody used to coat the wells of the plates might contribute to the differences in performance between the two ELISAs [58]. A recent study on 288 stool samples of children in a community village in Budhni, Peshawar, using *Entamoeba* CELISA PATH showed diagnostic sensitivity and specificity of 27% and 98.4% respectively, compared to microscopy-positive *Entamoeba* species [34]. Meanwhile, Pillai et al. [36] evaluated the usefulness of Optimum S kit to detect *E. histolytica* in 72 stool samples positive for *E. histolytica*/*E. dispar* complex; it showed only 4.2% sensitivity as compared to the combined results of two other coproantigens ELISAs.

Besides the commercially available kits, several laboratory based-assays have been developed using monoclonal and polyclonal antibodies against various *E. histolytica* antigens such as lipophosphoglycan, lectin-rich surface antigen and pyruvate phosphate dikinase [PPDK] [54, 59, 60]. In addition, a 170-kDa amoebic adherence lectin was reportedly detected in saliva of amoebiasis patients [61].

Table 1 Commercially available antigen and antibody detection assays, and rapid diagnostic tests for intestinal and extraintestinal amoebiasis

Assay	Specimen	Species detected	Diagnosis	Sensitivity (%)	Specificity (%)	Manufacturer
Antigen detection test						
TechLab *E. histolytica* II	Stool	*E. histolytica*	Intestinal Amoebiasis	96.9–100 (Manufacturer) 95[a] [13] 87.5[b] [26]	94.7–100 (Manufacturer) 93 [a] [13] 100[b] [26]	TechLab, Blacksburg, VA
				14.2[c] [29]	98.3[c] [29]	
				79[d] [30]	96[d] [30]	
			Carrier	71[e] [31]	100[e] [31]	
				20[f] [32]	86.7[f] [32]	
				60[g] [33]	93.4[g] [33]	
Entamoeba CELISA-PATH	Stool	*E. histolytica*	Intestinal Amoebiasis	95–100[b] (Manufacturer) 27.8[h] [34]	93–100 [b] (Manufacturer) 98[h] [34]	Cellabs Pty Ltd., Brookvale, Australia
Optimum S kit	Stool	*E. histolytica*	Intestinal Amoebiasis	100 [29]	Not published	Merlin Diagnostika, Berheim-Hersel, Germany
ProSpecT *Entamoeba histolytica* microplate assay	Stool	*E. histolytica*/ *E. dispar*	Intestinal Amoebiasis	87 (Manufacturer) 54.5[i] [29]	99 (Manufacturer) 94[i] [29]	REMEL Inc., Lenexa, KS
				78[i] [35]	99[i] [35]	
Antibody detection test						
IHA Cellognost-Amoebiasis	Pus	*E. histolytica*	Extraintestinal amoebiasis	100 [36] 99 [37]	90.9–100 [36] 95 [37]	Dade Behring Marburg GmbH, Germany)
Novagnost *Entamoeba* IgG		*E. histolytica*	Extraintestinal amoebiasis	> 95 (manufacturer) 95 [38]	> 95 (manufacturer) 98 [38]	(NovaTec Immundiagnostica, Dietzenbach, Germany
Bichro-Latex Amibe		*E. histolytica*	Extraintestinal amoebiasis	93.3 [39] 98.3 [40]	95.5 [39] 96.1 [40]	Fumouze Diagnostics, Levallois-Perret, France
				75 [41]	98.1[41]	
I.H.A. Amoebiasis		*E. histolytica*	Extraintestinal amoebiasis	93.4 [39] 78.6 [41]	97.5 [39] 96.7 [41]	Fumouze Diagnostics, Levallois-Perret, France
Amoebiasis Serology microwell II EIA		*E. histolytica*	Extraintestinal amoebiasis	97.9 [37] 92.5 [40]; 92 (Manufacturer)	94.8 [37] 91.3 [40]; 100 (Manufacturer)	LMD Laboratories, Inc., CA, USA
RIDASCREEN IgG *Entamoeba*		*E. histolytica*	Extraintestinal amoebiasis	100 (Manufacturer) 97.7–100 [42]	95.6 (Manufacturer) 97.4 [42]	R-Biopharm AG, Darmstadt, Germany
				69 [43]	90 [43]	
				56.4 [44]	92.1 [44]	
Rapid diagnostic test for intestinal amoebiasis						
Triage Parasite Panel (TPP)		*E. histolytica*	Intestinal amoebiasis	96–100[j] [45, 46] 68.3[k] [36]	99.1–100 [j] [45, 46] 100 [k] [36]	Biosite Diagnostic Inc., San Diego, CA, USA
				100[l] [47]	91.5[l] [47]	

Table 1 (continued)

Assay	Specimen	Species detected	Diagnosis	Sensitivity (%)	Specificity (%)	Manufacturer
RIDA®QUICK *Cryptosporidium*/*Giardia*/*Entamoeba* Combi		*E. histolytica*/*E. dispar*	Intestinal amoebiasis	100 [48] 62 [49]	80–88 [48] 96 [49]	R-BioPharm, Darmstadt, Germany
ImmunoCard STAT!® CGE		*E. histolytica*	Intestinal amoebiasis	88[m] [50]	100 [m] [50]	Meridian Bioscience Inc., Luckenwalde, Germany
RIDA Quick Entamoeba test				28.6[p] [51]	86 [p] [51]	R-Biopharm AG, Darmstadt, Germany
E. histolytica Quik Chek		*E. histolytica*	Intestinal amoebiasis	100[n] [52] 97[o] [53]	100 [52] 100 [n] [53]	TechLab, Blacksburg, VA, USA
Prototype of lateral flow dipstick test			Intestinal amoebiasis	65.4[q] [54]	92[q] [54]	

[a] Compared to culture and microscopy

[b] Compared to isoenzyme analysis

[c] Sensitivity and specificity compared to culture and microscopy

[d] Compared to real-time PCR

[e] Compared to real-time PCR

[f] Compared to microscopy

[g] Compared to microscopy

[h] Compared to microscopy

[i] Compared to microscopy (wet mounts and concentration)

[j] Compared to microscopy

[k] Compared to ProSpecT *Entamoeba histolytica* microplate assay

[l] Compared to microscopy

[m] Compared to real-time PCR

[n] Compared to ELISA antigen

[o] Compared to ProSpecT *Entamoeba histolytica* microplate assay

[p] Compared to Techlab *E. histolytica* II

[q] Compared to real-time PCR

Molecular diagnosis

Molecular diagnostic tests have gained primacy for detection of various infectious diseases, including amoebiasis [62]. There are different variants of DNA amplification techniques for differentiation and detection of the *Entamoeba* species in stools, tissues and liver lesion aspirates. They include conventional PCR, nested PCR, real-time PCR, multiplex PCR and loop-mediated isothermal amplification assay (LAMP) [63–67]. Table 2 shows the various assay types and parameters used in molecular diagnosis of amoebiasis.

Conventional PCR

To date, many genes are targeted for recognition and discrimination of the three *Entamoeba* species, i.e. small subunit rRNA, gene encoding a 30-kDa protein, DNA highly repetitive sequences, haemolysin gene (HLY6), cysteine proteinase, serine-rich *E. histolytica* (SREHP) gene, actin gene and tandem repeats in extrachromosomal circular DNA [68, 69, 81, 91, 92]. The HLY6 gene has been used to develop a PCR assay for the detection of *E. histolytica* DNA in stool samples, and showed 100% diagnostic sensitivity and specificity [69]. However, PCR assay targeting small subunit rRNA is most commonly used due to its presence in multiple copies of the extrachromosomal plasmids [93]. In addition, due to a high genetic variation between 18S rRNA gene of *E. histolytica* and *E. dispar*, it can be used to differentiate between these two species [92, 94].

A PCR targeting the small subunit rRNA gene has been developed to detect *Entamoeba* species in stool samples [70]. Seven out of 27 microscopy-positive stool samples were successfully identified by PCR in a Thai population. In Indonesia, the same gene target was used in a multiplex PCR performed on 30 samples of diarrheic stools. It showed 12 positive results, from which seven were positive for *E. histolytica*, two for *E. moshkovskii*, and three showed mixed infection of *E. histolytica* and *E. moshkovskii*, and no positive result for *E. dispar* [71]. A molecular epidemiology study among North East Indian population showed that the overall prevalence with any of the three morphologically indistinguishable *Entamoeba* species was 23.2% (95% CI = 20.9%, 25.6%). Of these, 13.7% (173/1260; 95% CI = 11.9, 15.7) and 11.8% (149/1260; 95% CI = 10.2, 13.8) of the subjects were infected with *E. histolytica* and *E. dispar*, respectively. The former group was PCR-positive either singly for *E. histolytica* or in combination with other intestinal protozoan parasites [95].

Nested PCR

Two nested PCR assays on DNA of stool samples targeting a 16S-like rRNA were reported by the International Centre for Diarrheal Diseases and Research, Dhaka, Bangladesh [72] and Jawaharlal Institute of Postgraduate Medical Education & Research, Puducherry, India [73]. They gave 100% diagnostic specificity and were able to distinguish infections caused by *E. histolytica*, *E. dispar* and *E. moshkovskii*. In a prevalence study in Malaysia, the nested PCR successfully detected 75% *E. histolytica*, followed by *E. dispar* (30.8%) and *E. moshkovskii* (5.8%) [74]. Another study on aborigines in Malaysia showed that 52 (80%, $n = 65$) microscopically positive samples were successfully amplified by the nested PCR. Of these, 65.4% were found to be *E. histolytica* and 13.5% were *E. dispar* and none was positive for *E. moshkovskii*. The nested PCR was also 100% specific as no amplification of other genomic DNA was observed [75]. In Mexico, a nested PCR was designed to detect and differentiate *E. histolytica* from *E. dispar* using a fragment of the *adh112* gene. Of 62 samples tested, 16.1% were positive for *E. histolytica* while none was positive for *E. dispar* [76]. Nevertheless, the identification of the *Entamoeba* species from stool specimens by nested PCR for individual species is a tedious process.

Nested multiplex PCR

In a trial to increase the sensitivity of PCR method, a nested multiplex PCR was developed for simultaneous detection of *Entamoeba* species using DNA extracted from stool specimens. Khairnar and Parija [96] found that the assay showed 94% sensitivity and 100% specificity. Fallah et al. [77] reported that the nested multiplex PCR was useful for the specific detection of pathogenic and non-pathogenic *Entamoeba* species in stool samples. Thirty-one (4.28%) out of 724 stool samples were positive for *E. histolytica*/*E. dispar*, with 54.8% samples positive for *E. dispar* and 8 (25.8%) samples positive for *E. histolytica*. In Egypt, a nested multiplex PCR detected samples positive of *E. histolytica* and *E. dispar* with a sensitivity of 96.8%. Of these samples, 17 (32.7%) were positive for *E. histolytica*, 12 (23.1%) for *E. dispar*, and 3 (5.7%) for both species. However, the nested multiplex PCR detected *E. dispar* in one of the negative control samples, thus giving a diagnostic specificity of 95%. In a study by ElBakri et al. [97], the nested PCR was used for simultaneous detection of *E. histolytica, E. dispar* and *E. moshkovskii* from 120 faecal samples collected from Sharjah Emirate, UAE. The result showed that 10% (12/120) samples were mono infected with *E. histolytica*; 2.5% (3/120) with *E. dispar*; and 2.5% (3/120) with *E. moshkovskii*. Furthermore, mixed infections by both *E. histolytica* and *E. dispar* were observed in 3.3% (4/120) samples; and *E. dispar* and *E. moshkovskii* in 0.8% (1/120) samples. Meanwhile, in Nigeria, the PCR results showed that out of 46 microscopy-positive samples, 16 (34.8%) successfully generated species-specific amplicons of *Entamoeba* species DNA. Infection with *E. dispar* (68.8%; 11/46) was the most common, followed by *E. histolytica* (37.5%; 6/46) and

Table 2 Assay types and parameters used in molecular diagnosis of intestinal and extraintestinal amoebiasis

Assay	Gene target	Amplification product (bp)	Name of primer	Primer sequence (5′-3′)	Name of probe	Targeted species	Sample	Country	Reference
Conventional PCR									
Conventional PCR	extra-chromosomal circular DNA of *E.histolytica*	125	P1	TCAAAATGGTCGTC GTCTAGGC		*E. histolytica*	pus of ALA	Pakistan, Sweden	[68, 69]
			P2	CAGTTAGAAATTAT TGTACTTTGTA					
	30-kDa antigen gene of pathogenic *E.histolytica*	100	P-11	GGAG GAGTAGGAAA GTTGAC					
			P-12	TTCTTGCAATTCCT GCTTCGA					
	HLY6 gene	256	Eh6F	GACCTCTCCTAATA TCCTCGT					
			Eh6R	GCAG AGAAGTACTG TGAAGG					
Single round PCR	small-subunit rRNA gene	166	EnF	ATG CAC GAG AGC GAA AGC AT	N/A	*E. histolytica*	Stool	Thailand	[70]
			EhR	GAT CTA GAA ACA ATG CTT CTC T					
		752	EnF	ATG CAC GAG AGC GAA AGC AT		*E. dispar*			
			Ehd	CAC CAC TTA CTA TCC CTA CC					
		580	EnF	ATG CAC GAG AGC GAA AGC AT		*E. moshkovskii*			
			Ehm	TGA CCG GAG CCA GAG ACA T					
Multiplex single round PCR			EntaF	ATG CAC GAG AGC GAA AGC AT	N/A	*E. histolytica*	Stool	Indonesia	[71]
			EhR	GAT CTA GAA ACA ATG CTT CTC T					
		752	EntaF	ATG CAC GAG AGC GAA AGC AT		*E. dispar*			
			EdR	CAC CAC TTA CTA TCC CTA CC					
		580	EntaF	ATG CAC GAG AGC GAA AGC AT		*E. moshkovskii*			
			EmR	TGA CCG GAG CCA GAG ACA T					

Table 2 (continued)

Assay	Gene target	Amplification product (bp)	Name of primer	Primer sequence (5′-3′)	Name of probe	Targeted species	Sample	Country	Reference
Nested PCR									
Nested PCR	16S-like RNA	900	E-1	TTT GTA TTA GTA CAA A	N/A	*E. histolytica* and *E. dispar*		Bangladesh, India	[72, 73]
			E-2	GTA [A/G]TA TTG ATA TAC T					
			Eh-1	AAT GGC CAA TTC ATT CAA TG					
			Eh-2	TTT AGA AAC AAT GCT TCT CT					
			Ed-1	AGT GGC CAA TTT ATG TAA GT					
			Ed-2	TTT AGA AAC AAT GTT TCT TC					
	SSU-rDNA gene of *E. moshkovskii* Laredo	258	Em1	CTC TTC ACG GGG AGT GCG		*E. moshkovskii*			
			Em-2	TCG TTA GTT TCA TTA CCT					
			nEm-1 nEm2	GAA TAA GGA TGG TAT GAC					
Nested PCR	16S ribosomal RNA	1496	E-1	TAA GAT GCA GAG CGA AA	N/A	Entamoeba genus (first PCR)	Stool, pus of ALA	Malaysia	[74, 75]
			E-2	GTA CAA AGG GCA GGG ACG TA					
		439	EH-1	AAG CAT TGT TTC TAG ATC TGA G		*E. histolytica*			
			EH-2	AAG AGG TCT AAC CGA AAT TAG					
		174	ED-1	TCT AAT TTC GAT TAG AAC TCT		*E. dispar*			
			ED-2	TCC CTA CCTATT AGA CAT AGC					
		553	Mos-1	GAA ACC AAG AGT TTC ACA AC		*E. moshkovskii*			
			Mos-2	CAA TAT AAG GCT TGG ATG AT					
Nested PCR-DGGE	*adh112* gene	268	Fw	GCA GAA AAA AAT AAT AAT AAC	N/A	Entamoeba genus (first PCR)	Stool	Mexico	[76]
			Rv	TTC ATT TGT TTT ACT TTC A					
			Fw	CGC CCG CCG CGC GGC CGC GGC		*E. histolytica*/*E. dispar*			

Table 2 (continued)

Assay	Gene target	Amplification product (bp)	Name of primer	Primer sequence (5′-3′)	Name of probe	Targeted species	Sample	Country	Reference
				CGG CCG GGG GCA CGC GGC GGC					
				AGA AAA AAA TAA TAA TAA					
			Rv	TTC ATT TGT TTT ACT TTC A					
Nested multiplex PCR	16S- like rRNA gene		E-1	TAAGATGCACGAGA GCGAAA	N/A	Entamoeba genus (first PCR)	Stool	India, Iran, Nigeria	[77–79]
			E-2	GTAC AAAGGGCAGG GACGTA					
		439	EH-1	AAGCATTGTTTCTA GATCTGAG		*E. histolytica* (second PCR)			
			EH-2	AAGA GGTCTAACCG AAATTAG					
		174	ED-1	TCTAATTTCGATTA GAACTCT		*E. dispar* (second PCR)			
			ED-2	TCCCTACCTATTAG ACATAGC					
		553	Mos-1	GAAA CCAAGAGTTT CACAAC		*E. moshkovskii* (second PCR)			
			Mos-2	CAATATAAGGCTTG GATGAT					
Real-time PCR									
Real-time PCR (Light Cycler)	18S rRNA	307	Eh-S26C	GTA CAA AAT GGC CAA TTC ATT CAA	LC-Red 640-TCG AAC CCC AAT TCC TCG TTA TCC	*E. histolytica*	Stool, pus of ALA	USA	[66, 80]
			Ed-27C	GTA CAA AGT GGC CAA TTT ATG TAA GCA		*E. dispar*			
			Eh/Ed-AS25	GAA TTG ATT TTA CTC AAC TCT AGA G	Eh/Ed-25-fluores-cein (fluorescein-GCC-ATC TGT AAA GCT CCC TCT CCG A X	*E. histolytica* and *E. dispar*			
Real-time PCR (Taq Man)	18S rRNA	172	Ehd-239F	ATTGTCGTGGC ATCCTAACTCA	VIC-5′-TCAT TGAATGAATT GGCCAT TT-3′-nonfluores-cent quencher	*E. histolytica*	Stool, pus of ALA	Netherlands, Egypt	[81–83]
			Ehd-88R	GCGGACGGCTCATT ATAACA					

Table 2 (continued)

Assay	Gene target	Amplification product (bp)	Name of primer	Primer sequence (5′-3′)	Name of probe	Targeted species	Sample	Country	Reference
Real-time PCR (Taqman)	small subunit rRNA gene	134	Eh-f	AACAGTAATAGTTT CTTTGGTTAGTA AAA	YYT, 5′-ATT AGT ACA AAC TGG CCA ATT CAT TCA-3′ (Eclipse)	*E. histolytica*	pus of ALA	Bangladesh	[63]
Real-time PCR (Molecular Beacon)	18 s rRNA	134	Ehf	AACAGTAATAGTTT CTTTGGTTAGTA AAA	Texas Red-GCGAGC--ATTAGTAC AAAATG GCCAAT TCATTC A-GCTCGC-dR Elle	*E. histolytica*	Stool, pus of ALA	Bangladesh	[30]
			Ehr	CTTAGAATGTCATT TCTCAATTCAT					
Real time PCR	16 s rRNA	134	Ehf	AACAGTAATAGTTT CTTTGGTTAGTA AAA	SYBR Green Supermix	*E. histolytica*	Stool	Mexico	[85]
			Ehr	CTTAGAATGTCATT TCTCAATTCAT					
Multiplex Real time PCR	small-subunit rRNA gene sequences	222	EhsmF EhdmR	CGAAAGCATTTCAC TCAACTG TCCCCCTGAAGTCC ATAAACTC	Ehdm-FL: 5′-ACTA TA AACGATGT CAAC CAAGGATT GGAT GAAA-FITC-3′	*E. histolytica*	Stool	Thailand	[86]
					Ehd-640: 5′-TCAG AT GTACAAAG ATAG AGAAGCAT TGTT TCTA-phosphat-e-3	*E. dispar*			
					Em-705: 5′-AAGA AA TTCGCGGA TGAA GAA ACATTGTT T-phosphate-3	*E. moshkovskii*			
Multiprobe real-time PCR	*E. histolytica* SSU rRNA	110	EntaTaq-L	GGACACATTTCAAT TGTCCTA	YAK-5'TGTAGTTA TCTAAT TTCGGT TAGACC-3′	*E. histolytica*	Stool	Taiwan	[58]
			EntaTaq-R	CATCACAGACCTGT TATTGCTG					
		111	EntaTaq-L	GGACACATTTCAAT TGTCCTA	FAM-5'TGTTAGTT ATCTAA TTTCGA TTAGAACTC-3'	*E. dispar*			
			EntaTaq-R	CATCACAGACCTGT TATTGCTG					

Table 2 (continued)

Assay	Gene target	Amplification product (bp)	Name of primer	Primer sequence (5′-3′)	Name of probe	Targeted species	Sample	Country	Reference
Loop-mediated isothemal amplification assay (LAMP)									
LAMP	small-subunit ribosomal DNA in the *E. histolytica* genome	447	Outer primer EHd1-F3	Ehd1-F3-AAAGATAA TACTTGAGACGA TCC		*E. histolytica*	Stool	Taiwan, India	[17, 87]
			Outer primer EHd1-B3	Ehd1-B3-TCGTTATC CGTTATAATCTT GG					
			Inner primer Eh1-FIP	Eh1-FIP GCATCCTA ACTCACTTAGAA TGTCAAGTACAA AATGGCCAATTC ATTC					
			Inner primer Ehd1-BIP	Ehd1-BIP-CACGA-CAATTGTAG AACACACAGTTC CTCGATACTACC AACTGAT					
LAMP	*E. histolyica* hemolysin gene, HLY 6		Outer primerEh-2F3	Eh-2F3- GCACTATA CTTGAACGGATT G		*E. histolytica*	Stool	Philippines	[88]
			Outer primer Eh-2B3	Eh-2B3-GTTTGACA AGATGTTGAGTG A					
			Inner primer Eh-2FIP	Eh-2FIP-TCGCCCTA TACTCAAATATG ACAAGACTTTGG TGGAAGATTCAC G					
			Inner primer Eh-2FIP	Eh-2FIP- ATCTAGTA GCTGGTTCCACC TGAACACCTAAT CATTATCTTTAC CAATC					
			Aditional primer Eh-2F2	Eh-2F2 -ACTTTGGT GGAAGATTCACG					
			Aditional primer Eh-2B2	Eh-2B2-CACCTAAT CATTATCTTTAC CAATC					
Stem LAMP	18S small subunit ribosomal RNA	207	F3	AAATACAAGGATAG CTTTGTG		*E. histolytica*	Stool	Kenya	[89]
			B3						

Table 2 (continued)

Assay	Gene target	Amplification product (bp)	Name of primer	Primer sequence (5′-3′)	Name of probe	Targeted species	Sample	Country	Reference
				AAGCTCCCTCTCCG ATGTC					
			FIP	CTCAATTCATTGAA TGAATTGGCATG ATAAAGATAATA CTTGAGAC					
			BIP	CAATGAGAATTTCT GATCTATCCGTT ATCCGTTATAAT CTTGG					
			LF	TTTGTACTAATACA AACTGGATC					
			LB	CAGTTGGTAGTATC GAGGAC					
			SF	CGACAATTGTAGAA CACACAG					
			SB	ATCCTAACTCACTT AGAATGTC					
LAMP-NAL-FIA	serine-rich *E. histolytica* protein (SREHP) gene		Eh-FIP-SER	GCTTCGTTCTTTAA AAATACACCGTC ATTCTTGATTTG GATCAAGAAGT		*E. histolytica*	pus of ALA	Malaysia	[90]
			Eh-BIP-SER-FITC	AGTA GCTCAGCAAA ACCAGAATCACT TGCTTTTTCATC TTCATCA					
			Eh-F3-SER	TGCATTCACTAGTG CAACT					
			Eh-B3-SER	GCTTGATTCTGAGT TATCACTTG					
			Eh-LB-SER-Biotin	AAGTTCAAATGA AGATAATGAA					
	Entamoeba spp. (LSU-rRNA gene		Eh-FIP-HLY	TACGCCATTTCGTT TCCTTACTCGAT TTCTTAACTGAT ACTCGACCG		*E. histolytica*			
			Eh-BIP-HLY-FITC	AGATTGAAACTGTC CTTAGTGCAGCA GTTCTAAGATGT TTTTTTCCTC					

Table 2 (continued)

Assay	Gene target	Amplification product (bp)	Name of primer	Primer sequence (5′-3′)	Name of probe	Targeted species	Sample	Country	Reference
			Eh-F3-HLY	CCTGAAAATGGATG GCATTA					
			Eh-B3-HLY	CCCTAATCCAAGTA ATGTTGTT					
			Enta-LB-HLY-Tex	CTTGGTGGTAGTAG CAAATACTAAG					

E. moshkovskii (18.8%; 3/46) [78]. A first molecular epidemiology study of *E. histolytica*, *E. dispar* and *E. moshkovskii* infection in Yemen using multiplex nested PCR revealed that 20.2%, 15.7% and 18.2% of 605 samples were positive for *E. histolytica*, *E. dispar* and *E. moshkovskii*, respectively [79]. These result suggested that nested multiplex PCR offers high diagnostic sensitivity and specificity, and allows simultaneous detection and differentiation between *E. histolytica* and *E. dispar* in microscopy-positive stool samples [70, 96].

Real-time PCR

Conventional and nested PCR are time consuming especially when processing many samples, costly, results are non-quantitative, and may produce false positive results due to carry-over contamination [3, 15]. Thus, real-time PCR assay has gained a lot of attention for laboratory diagnosis of infection since it can enhance diagnostic sensitivity, eliminate post-PCR manipulation and minimize contamination [3]. It also allows quantification of the relative number of parasites present in various forms of clinical samples including stool, liver abscess, aspirate and urine [96]. Many studies have been published on the use of real-time PCR assays for specific detection of *E. histolytica* and *E. dispar* using DNA from stool samples [30, 66, 80, 81, 98]. Most of them targeted either 18S rRNA gene or species-specific episomal DNA repeat genes. For example, Qvarnstrom et al. [80] compared real-time PCR using different probes, and the results suggested that the TaqMan method, which targets the 18S rRNA gene, was more specific than the SYBR Green approach for diagnosis of amoebiasis. They also reported that the probe-based real-time PCR assays can be used to detect *E. histolytica* in clinical samples with very low number of parasites which are not detectable by conventional PCR. In Mexico, analysis of stool samples from 273 children using Faust stool concentration technique showed that 25 (9.2%) were positive for *E. histolytica/E. dispar/E. moshkovskii*. Of these, 3 were positive for *E. histolytica* by SYBR Green real-time PCR; and 2 samples that were negative for *E. histolytica/E. dispar/E. moshkovskii* by the Faust technique were positive by the real-time PCR [85]. Meanwhile, in another study, 672 stool samples from endemic areas in Vietnam and South Africa were used to evaluate a real-time PCR targeting 310 bp fragment of rDNA-containing amoeba episome. The results were compared with those from amoeba culture and microscopy [66]. They reported that all samples positive by microscopy and 88% samples positive by culture were also positive by real-time PCR. When compared to culture and isoenzyme analysis, the real-time PCR was 100% specific for detection of both *Entamoeba* species. Roy et al. [30] showed that real-time PCR was 99% sensitive and specific for detection of *E. histolytica* in stool as compared to conventional PCR. Another recent study from Egypt used nested multiplex PCR and TaqMan real-time PCR to determine the prevalence of *E. histolytica.* Among 194 microscopy-positive *Entamoeba* samples, the nested PCR identified 8.7% ($n = 17$) as *E. dispar* and 10.3% (20/194) as *E. histolytica*. With the real-time PCR, 5.7% (11/194) and 9.8% (19/194) samples were found to be positive for *E. dispar* and *E. histolytica* respectively [82].

Multiplex real-time PCR

Recent developments in multiplex real time PCR make it possible to rapidly identify, genotype and quantify multiple DNA targets simultaneously in a single reaction. In Thailand, a multiplex real-time PCR was established to differentially detect *E. histolytica*, *E. dispar* and *E. moshkovskii* [86]. The assay detected *E. histolytica* in four of 32 microscopy-positive stool samples. Most of the stool samples were positive for *E. dispar*, and one sample had mixed infection with *E. moshkovskii*. Meanwhile, Liang et al. [58] reported the use of a single-tube multiprobe real-time PCR (EntaTaq) assay for simultaneous detection of *E. histolytica* and *E. dispar*. The assay identified 23.5% (12/51) *E. histolytica* and 41% (16/39) *E. dispar* in samples which were negative by nested PCR, without cross reactivity with other commensal protozoa. Based on the data of previous studies, the detection limit of the EntaTaq was 10 times greater than nested PCR (10–100 trophozoites/ml) [17]. In another study, stool samples were analyzed from 396 Egyptian patients with diarrhoea, along with 202 samples from healthy controls. A total of 43 patient samples were microscopy-positive for *E. histolytica/dispar*; however, a real-time PCR assay only detected eight samples with *E. dispar* while *E. histolytica* was not detected at all [83]. Thus, the use of the real-time PCR for simultaneous detection of multiple DNA targets would be beneficial for the accurate and rapid diagnosis of amoebiasis.

Currently, several multiplex PCR panels have been certified as in vitro diagnostic tests (IVD), allowing the simultaneous detection of multiple pathogens in stool samples [99, 100]. Table 3 illustrates the performances of commercially available rapid PCR-based tests for the detection of *E. histolytica* in stool samples. In one study using real-time multiplex PCR, the RIDA GENE Parasite stool panel II (R-Biopharm, Darmstadt, Germany) was evaluated for the specific detection of *E. histolytica*, *Giardia lamblia* and *Cryptosporidium parvum* in 180 patients suffering from diarrhoea who attended the outpatient clinic of a central teaching hospital for children in Baghdad city, Iraq. These results showed 100% sensitivity and specificity when compared to microscopy for the detection of *E. histolytica* [101]. A study conducted by Laude et al. [105] using 185 stool samples collected from 12 parasitology laboratories in France reported that the G-DiaParaTrio® (Diagenode Diagnostics, Belgium) multiplex PCR assay identified 38 samples with

Table 3 Performances of commercially available rapid PCR-based tests for detection of *Entamoeba histolytica* in stool specimens

PCR method	Type assay	Gene target	Sensitivity (%)	Specificity (%)	Manufacturer	Reference
RIDA GENE Parasiter Stool Panel	Real-time multiplex PCR	18 s-ITS	100	100	R-Biopharm, Darmstadt, Germany	[101, 102]
G-DiaParaTrio®	Multiplex PCR	N/A	100	100	Diagenode Diagnostics, Belgium	[103]
xTAG GPP assay	Multiplex PCR	N/A	100;	89	Luminex Molecular Diagnostics, Austin, TX, USA	[104]
FilmArray Gastrointestinal Panel	Nested multiplex PCR	N/A	100	100	BioFire Diagnostics, Salt Lake City, UT, USA	[102]

N/A data not available

G. intestinalis, 25 with *C. parvum/C. hominis* and 5 with *E. histolytic,* leading to sensitivity/specificity of 92%/100%, 96%/100% and 100%/100% for *G. intestinalis*, *C. parvum/C. hominis* and *E. histolytica*, respectively. A recent study conducted on 172 clinical stool samples using multiplex PCR ParaGENIE G-Amoeba (Ademtech, France) reported a high sensitivity and specificity for the detection of *G. intestinalis* and *E. dispar*/*E. moshkovskii* from stools (89.7%/96.9% and 95%/100%, respectively). Although two false-negative samples were observed which were confirmed to be positive for *E. histolytica* by an external PCR assay, the multiplex PCR showed better specificity compared to an antigen detection test (ELISA) [104]. The Luminex xTAG gastrointestinal pathogen panel assay (GPP) (Luminex Molecular Diagnostics, Austin, TX, USA) is another commercial qualitative multiplex test, it able to identify 19 enteric pathogens in one reaction in 6 h. The test sensitivity and specificity for detection of *E. histolytica* from clinical specimens was reported to be 100% and 89%, respectively as compared to real time PCR [103]. Although real-time PCR assay is highly sensitive and specific, the major disadvantage of this method is its relative high cost, thus may not be utilized by most laboratories in developing countries [106].

Loop mediated isothermal amplification assay (LAMP)

The use of isothermal amplification assay is a good choice for molecular diagnosis especially for low resource areas due to its high sensitivity, specificity, rapidity and simplicity [107, 108]. Liang et al. [17] have developed a loop-mediated isothermal amplification assay (LAMP) assay for intestinal amoebiasis and compared the results with a nested PCR. *E. histolytica* DNA was detected in 60% samples (18/30) using LAMP and 33% samples (10/33) using nested PCR, thus showing the superior sensitivity of the former. In a study conducted by Singh et al. [87], the LAMP assay was found to be more sensitive than conventional PCR assay for diagnosis of ALA. Out of 50 pus samples, 36 (72%) were positive for *E. histolytica* by conventional PCR assay and 41 (82%) by LAMP assay. However, a study by Rivera and Ong, [88] in the Philippines found that the detection limit of the LAMP assay was five parasites per reaction, which corresponded to approximately 15.8 ng/μl DNA, while the detection limit for conventional nested PCR was 2 ng/μl DNA samples. A modification of the isothermal assay based on 18S small subunit ribosomal RNA gene and designed with extra reaction accelerating primers is called stem LAMP. Mwendwa et al. [89] used the stem LAMP and detected 36 of 126 (28.6%) DNA samples as *E. histolytica*, and this was better when compared to the standard LAMP test (15.9%, 20/126) or conventional PCR (13.5%, 17/126). In another study, a four target nitrocellulose-based nucleic acid test using lateral flow immunoassay biosensor that detected amplicons from a thermostabilized triplex LAMP assay was developed. The biosensor detection limit was 10 *E. histolytica* trophozoites and showed 100% specificity when evaluated against three medically important *Entamoeba* species and 75 other pathogenic microorganisms [90].

Rapid diagnostic test

In recent years, there is emergence of rapid diagnostic tests as point-of-care (POC) tests for amoebiasis [109]. Such a test represents a bridge that can connect the laboratory and the field. It allows for the mass screening of endemic populations and for the monitoring of control programmes, thereby providing quick diagnosis and reducing disease transmission. It also provides rapid result to a physician, thus shortening the duration gap between disease diagnosis and treatment [110]. As compared to the other laboratory tests such as ELISA and PCR, rapid test avoids the need of expensive equipment. Thus, it is a preferred diagnostic tool in developing countries with low resources [111].

There are a number of rapid diagnostic tests available in the market for intestinal amoebiasis (Table 1). The Triage Parasite Panel (TPP) (Biosite Diagnostic Inc., San Diego, CA, USA) is the first lateral flow test to simultaneously detect antigens specific for *G.lamblia, E. histolytica*/*E. dispar* and *C. parvum*. The test uses monoclonal antibodies specific to a

29-kDa surface antigen of *E. histolytica/E. dispar, G. lamblia* alpha-1-giardin, and *C. parvum* protein disulfide isomerase. Two studies showed that the TPP kit had high diagnostic sensitivity (96% to 100%) and specificity (99.1% to 100%) for detection of *E. histolytica/E. dispar*, when compared to microscopy [45–47]. In contrast, Garcia et al. [45] and Pillai and Kain [36] found that the sensitivity of the kit was low (68.3%), albeit with high specificity (100%) when compared with ProSpecT test. This is corroborated by the work by Leiva et al. [112], who found that the sensitivity of TPP kit was low when compared to PCR assay. In addition the kit is unable to differentiate among *E. histolytica*, *E. dispar* and *E. moshkovskii*. Furthermore, either fresh or fresh-frozen non-preserved stools should be used with the TPP kit which may be impractical in some situations [3].

A retrospective study was performed by Van den Bossche and his colleagues [48] to evaluate the lateral flow RIDA®QUICK Cryptosporidium/Giardia/*Entamoeba* Combi (R-BioPharm, Darmstadt, Germany), using stool samples collected from patients at an outpatient clinic of the Institute of Tropical Medicine (ITM), Antwerp, Belgium or the Central Laboratory of Clinical Biology (CLKB). The kit demonstrated 100% sensitivity, while the specificity ranged from 80% to 88% for detection of *E. histolytica*. This result differed from Goñi et al. [49], which showed that the kit exhibited 62% sensitivity and 96% specificity for *E. histolytica*. The lower specificity in the study by Van den Bossche et al. [48] can be explained by the high number of *E. dispar* samples, which substantially influences specificity since this kit is unable to differentiate between *E. histolytica* and *E. dispar* [15]. Another version of the one-step immunochromatographic test for the qualitative detection of *C. parvum*, *G. lamblia* and *E. histolytica* antigens in human stool samples has been introduced to the market by Meridian Bioscience Inc. (Luckenwalde, Germany) and is known as ImmunoCard STAT!® CGE. This new ImmunoCard rapid antigen detection test exhibited 88% sensitivity and 92% specificity as compared to real-time PCR in detection of *E. histolytica*, but it showed cross-reactivity with *E. dispar* [50]. A study was conducted on diarrheic/dysenteric stool samples from clinically suspected individuals from Beni-Suef, Egypt, using RIDA®QUICK *Entamoeba* Test (R-Biopharm AG, Darmstadt, Germany), an immunochromatographic (ICT) rapid assay for the qualitative determination of *E. histolytica* / *dispar*, and Techlab *E. histolytica* II ELISA test was used as the reference. Of 7 specimens that were positive by the ELISA, only 2 specimens were positive by the ICT, thus a sensitivity of 28.6%, and the specificity was reported as 86.1% [51].

The third generation of a rapid test known as *E. histolytica* Quik Chek (TechLab, Blacksburg, VA, USA) was recently introduced to the market. The antibody used in this kit is specific against the *E. histolytica* adherence lectin [113]. The test is a modified version of the TechLab *E. histolytica* II ELISA, but uses a flow-through format. An evaluation of this point-of-care rapid test in a cohort of children in Bangladesh showed 100% sensitivity and specificity when compared to an ELISA antigen detection assay [52]. When compared to ProSpecT microplate assay, this kit exhibited 97% sensitivity and 100% specificity [53]. These findings indicated that the new Quik Chek assay was robust and can specifically detect *E. histolytica* trophozoites in unfixed, frozen clinical stool samples. However, it requires an additional incubation of both conjugate and chromogen which increases the processing time. Furthermore, it requires cold-chain transportation.

Our group previously reported the development of a lateral flow dipstick test which detected *E. histolytica* PPDK in stool samples [54]. When compared to real-time PCR, the diagnostic sensitivity of the dipstick was 65.4% ($n = 17/26$), while the specificity when tested with stool samples containing other intestinal pathogens was 92% (23/25). Although not highly sensitive, it was superior to the performance of Techlab *E. histolytica* II ELISA which detected only 19.2% (5/26) of the same set of PCR-positive samples. Thus, the lateral flow dipstick test showed good potential to be further developed into a stool rapid test for intestinal amoebiasis.

Extraintestinal amoebiasis

Clinical manifestations of ALA are highly variable, thus making the diagnosis difficult [56, 114]. Ultrasound, computed tomography and magnetic resonance are very useful techniques and have excellent sensitivity for detection of liver abscess arising from any cause [115]. However, these detection methods cannot distinguish amoebic abscesses from pyogenic abscesses or necrotic tumours. Most patients with ALA do not have co-existing amoebic colitis. Only less than 10% of amoeba have been identified in stool samples from ALA patients using microscopy and antigen detection tests [116]. Consequently, stool microscopy or antigen detection tests are not helpful for diagnosis of ALA [27, 56, 61].

Microscopic examination

ALA can be confirmed by microscopic examination of liver pus to look for amoebic trophozoites [117]. However, aspiration of liver pus in an ALA patient is not necessary for establishing the diagnosis. When aspirated, they contain acellular debris that forms a brown, thick fluid (‘anchovy paste’). Although the presence of amoebic trophozoites in aspirated pus is confirmatory for ALA, it is not a sensitive diagnostic method. Trophozoites are seen in a minority of aspirates (< 20%) and typically only seen when the wall of the cyst is sampled [118, 119]. Hence, it is not surprising that many authors reported their total absence or very low incidence on examination of liver pus. In general, trophozoites have been seen in aspirated pus in 11–25% cases [120]. Parija and

Khairnar [121] found trophozoites of *E. histolytica* in pus in 7.2% cases, while Haque et al. [56] showed that 11% of aspirated liver pus samples were positive for the organism.

Microscopic examination of the pus may show dead and deformed hepatocytes, red blood cells and some polymorphs. A good staining method is necessary to visualize the morphological changes in the liver tissue and also differentiates the amoebas against the surrounding cells. The common staining techniques are haematoxylin and eosin (H&E), periodic-acid Schiff (PAS) and immunostaining. With H&E and PAS stains, amoebic trophozoites are difficult to differentiate from macrophages because of similarities in size and morphology [122]. Examination of fixed and stained biopsy samples using H&E stain is challenging due to lack of differentiation between the stained trophozoites and surrounding tissues [123]. With PAS stain, the amoebae are intensely PAS positive due to the presence of glycogen in cytoplasm; however, liver cells also contain glycogen [124]. Moreover, these two staining methods require high technical expertise to identify and interpret the results. In comparison, immunohistochemistry (IHC) is presumed to be more specific as it is the consequence of specific reactions between amoebic antigens and antibodies against them. Although IHC is still rarely reported for use in investigation of invasive amoebiasis [122], it is potentially very useful as a confirmatory test for ALA when liver pus aspirate biopsy or autopsy is available.

Antibody detection test

Petri and Singh [60] reported that anti-amoebic antibodies were detectable in more than 90% of patients with ALA within 7 to 10 days after symptoms began [117]. Therefore, serology is used for diagnosis of ALA, in combination with observations from clinical manifestations and result of radiological imaging. Several assays are commonly used to detect the anti-amoebic antibodies, such as ELISA, indirect haemagglutination (IHA), indirect immunofluorescence assay (IFA), latex agglutination, immunoelectrophoresis, counterimmunoelectrophoresis (CIE), amoebic gel diffusion test, immunodiffusion and complement fixation test [125]. Of these, ELISA is the most popular technique and has been used to investigate the epidemiology of symptomatic amoebiasis due to its reliability and ease of performance [113, 126].

A study was performed to compare the performance of several antibody detection assays on patients in Kuwait suspected of ALA using IHA (Collegnost Amoebiasis, Germany), Amoebiasis Serology Microwell II EIA (LMD LAboratories, CA) and ImmunoTab test (Institute Virion, Germany). The true positive cases were defined as patients positive by CT scan or ultrasonography with an IHA titer ≥ 1:256. The sensitivity and specificity of the IHA, ImmunoTab and Microwell II EIA was found to be 99% and 99.8%, 98% and 95.5% and 97.9% and 94.8%, respectively [37]. In other studies, the sensitivity of the Microwell II EIA for the detection of specific *E. histolytica* antibodies in ALA patients was reported to be 97.6–99% [125, 127].

Hung et al. [128] reported that IHA showed high specificity (99.1%) when tested with samples from patients with gastrointestinal symptoms, including HIV-infected patients. However, it showed lower sensitivity when compared to ELISA due to false-negative results [129]. In a village in West Kalimantan, Borneo, the seroprevalence of anti-amoebic antibodies among the population with IHA titers equal or greater than 1:128 was found to be 7% [130]. Meanwhile, the seroprevalence among blood donors from Kelantan, Malaysia, was reported to be 16% [131]. A study conducted by Dhanalakshmi et al. [43] using IHA, based on a recombinant calcium binding domain-containing protein, on serum of suspected amoebiasis patients showed 62% sensitivity and 96% specificity. In comparison, an antigen detection ELISA on the same set of samples showed 69% sensitivity and 90% specificity.

Garcia et al. [132] reported that the IFA was a reliable, reproducible and a rapid tool to differentiate ALA from other non-amoebic etiologies. In addition, it helps to differentiate current and past (treated) infected patients. The sensitivity of IFA was reported to be 93.6% (higher than ELISA) and the specificity was 96.7% [133]. However, this technique is difficult to perform routinely since it requires skill to culture the parasite and prepare the antigen [134].

The common commercially available antibody assays for detection of *E. histolytica* antibodies in human serum are included in Table 1. Most of these commercial tests are coated with crude soluble trophozoite antigen for the detection of anti-amoebic antibodies. Several studies have demonstrated high sensitivity of crude soluble and excretory-secretory antigens in detecting amoebic antibodies in ALA patients [38–40, 42, 135, 136, 137]. However, the drawbacks using crude antigens are the high cost and tediousness of maintaining *E. histolytica* culture, and other problems associated with the mass production of the antigen. In addition, the crude amoebic antigen gave false positive results, thus decreasing the test specificity [138]. In endemic settings, it tends to produce high background readings; thus, the antibody cut-off titre may be needed to be adjusted from that recommended by the manufacturer. In general, the use of the current commercial antibody detection tests is only reliable in developed countries where amoebiasis is not endemic. However, if the vast majority of patients in these countries are immigrants from developing countries, the usefulness of this test in developed countries may also be doubtful [60]. In endemic areas, individuals are constantly exposed to *E. histolytica*, and therefore the available IgG antibody detection tests are unable to definitively distinguish between past and current infections [27, 139]. The test may remain positive in patients after a few years of infection, and thus is a major problem that must be addressed.

To overcome this limitation, a standardized serological test based on a well-defined antigen is required. A heavy subunit of *E. histolytica* lectin (152 kDa) and PPDK (110 kDa) for serodiagnosis of ALA have been shown to possess diagnostic sensitivities of above 80% and no cross-reactivity in Western blot analysis [137]. The recombinant form of the PPDK has been produced and Western blot using a panel of serum samples probed with horseradish conjugated anti-human IgG4 showed a high diagnostic sensitivity (93.3%) and specificity (100%), when compared to blots using IgG and IgG1 as secondary antibodies [140].

Other recombinant proteins that has been used as antigens in amoebiasis serology included serine rich protein, 170 kDa subunit galactose-specific adhesin, cysteine proteinase, putative alcohol dehydrogenase and phosphoglucomutase [141–143, 144, 145].

Molecular diagnosis

Table 2 includes information on the assays that have been used in detecting *E. histolytica* DNA in liver abscess/pus samples. Detection of ALA by conventional PCR showed varied diagnostic sensitivity, ranging from 33% to 100% [56, 68, 121, 125, 146]. When PCR using specific primers for *E. histolytica* 18S rDNA was used to detect the parasite in liver pus samples, only 33% sensitivity was recorded; whereas 100% sensitivity was obtained when a second primer pair specific for a gene encoding a 30-kDa antigen was used [125, 147]. Zaman et al. [68] was successful in obtaining 100% diagnostic sensitivity by using two pairs of published primers namely P1-P2 and P11-P12 which targeted the extrachromosomal circular DNA of *E. histolytica* and the 30-kDa antigen gene, respectively. In another study, PCR was performed which targeted *E. histolytica* HLY6 gene using pus samples from ALA patients. The hemo-PCR detected 89% of the samples, as compared to 77% and 28% detection when PCR assays were performed using primers based on the 30-kDa antigen gene and 18S rDNA, respectively [69]. In Canada, a total of 25 ALA samples were assessed by microscopy, serology, and nested PCR. The results showed that microscopy was negative for *E. histolytica* in all samples, and nested PCR as well as serology were positive in 11 samples [148].

In another study, Parija and Khairnar [121] reported that a nested multiplex PCR detected *E. histolytica* DNA in 100% ($n = 37$) liver abscess pus samples from ALA patients collected prior to metronidazole treatment, as compared to only 70.6% (53/75) after therapy. The decline in diagnostic sensitivity can be attributed to the parasite DNA clearance from the abscess subsequent to the parasite destruction post-treatment. However, pus aspiration carries the risk of bacterial infection or spillage of abscess content; thus, it is performed only if there is a danger of rupture of the abscess or if the size of abscess is greater than10 cm in diameter. Abscess should also be drained if it does not respond to medical therapy within 3 to 5 days [149, 150]. In addition, it was reported that the PCR detected *E. histolytica* DNA in 21 of 53 (39.6%) urine specimens of ALA patients. Among them, four of 23 (17.4%) urine specimens were collected prior to metronidazole treatment, and 17 of 30 (56.7%) were collected after treatment [121]. A study conducted in Malaysia showed that real-time PCR successfully detected *E. histolytica* DNA in 76.7% of 30 liver abscess samples, whereas IHA detected the presence of anti-*E. histolytica* antibodies in 46.7%(14/30) of the corresponding serum samples [56]. Meanwhile, a study conducted in Bangladesh by Haque et al. [63], using real-time PCR on 98 ALA patients showed that the assay was able to detect *E. histolytica* DNA in 49%, 77% and 69% of blood, urine and saliva samples, respectively. Ahmad et al. [84] showed that *E. histolytica* detection by real-time PCR had higher diagnostic sensitivity than antigen detection method. In a study by Roy et al. [30], a real-time PCR assay on liver pus samples utilizing a molecular-beacon probe was found to be comparable to the TaqMan-based method by Blessmann et al. [66].

Weitzel et al. [102] reported the first use of Rida®Gene Parasitic Stool Panel (R-Biopharm, Darmstadt, Germany) and FilmArray Gastrointestinal Panel (BioFire Diagnostics, Salt Lake City, UT, USA) on samples from aspirated pus of patients with cystic focal liver lesions. Both commercial kits confirmed the diagnosis of ALA within a short time, with FilmArray system giving a faster result than the Rida®Gene test (1 h vs. 3 h) and required less hands-on time (5 min vs. 45 min).

Rapid diagnostic test

To date, there is no commercial rapid diagnostic test for the diagnosis of ALA. However, a proof-of-concept of a lateral flow dipstick test for rapid detection of ALA has been developed. The test is based on detection of anti-PPDK IgG4 antibody in infected patients. Initial evaluation of the rapid test showed 87% diagnostic sensitivity and 100% specificity [140]. Recently, an immunochromatographic test using fluorescent silica nanoparticles coated with C-terminal region of the intermediate subunit of *E. histolytica* Gal/GalNAc lectin protein has been reported. The kit showed 100% sensitivity and 97.6% specificity when tested with sera from healthy controls and patients with other infectious diseases [151]. Both of the above tests seemed to have good potential for rapid diagnosis of ALA and merit further multicentre validation using a much larger sample size.

Future perspectives

Quick and correct diagnosis of amoebic infection is important to avoid morbidity, death and disease transmission. Since most endemic areas are underdeveloped and lack resources

(financial, facility, skilled manpower), tests which fulfilled the World Health Organization ASSURED criteria would be welcomed, i.e. affordable, highly sensitive and specific, user-friendly, robust and rapid, equipment-free and deliverable to those who need them [111]. Thus far, the tests developed for amoebiasis fulfilled some but not all of the above criteria. Thus, scientists working on laboratory diagnosis of amoebiasis should try to develop tests that fully comply with the above criteria.

Other than identifying infected individuals, it would be very useful to identify biomarkers that can predict individuals who are susceptible to acquire symptomatic infection, distinguish between the different stages of an infection, and monitor whether treatment leads to cure. The combination of well-characterized patient samples and advances in 'omics' technologies may make this possible. Genomics technologies may be able to assess host susceptibility based on the variations in single nucleotide polymorphism (SNP) of a certain loci [152]. Meanwhile, proteomics and metabolomics may help to distinguish the different stages of *E. histolytica* infection. Massive proteomic data on amoeba, especially *E. histolytica*, have been generated, whereby its proteome and sub-proteomes have been explored [153–158]. Hence, the biological processes of the *E. histolytica* proteins could be deduced. This will help us to better understand the role of certain *E. histolytica* proteins in the pathogenesis even though not all proteins are well characterized. Using quantitative proteomic, some *E. histolytica* proteins of a virulent variant have been confirmed to be differentially abundant when compared to its non-virulent variant or other non-pathogenic *Entamoeba* species [159, 160]. In addition, a comparison between *E. histolytica* life stages has been made using this approach [152]. Proteins that showed increased in abundance during certain life stages can be explored as potential biological markers for *E. histolytica* diagnosis, as well for drug and vaccine development.

Conclusion

Laboratory diagnosis of both intestinal and extraintestinal amoebiasis has improved with more sensitive and specific tests. For intestinal infection, molecular diagnostic is the way forward, with PCR-based assays for well-equipped laboratories and LAMP for low-resource settings. For extraintestinal infection, improvement in serodiagnosis is needed to accurately detect active infection since the exclusion of past infection is a challenge for the current IgG-based assays. Similarly, the sensitivity of rapid POC diagnostic tests for ALA can also be further improved and be made commercially available to enable its wider application. Further research areas that can be explored include discovery of biomarkers that can predict individuals prone to symptomatic infection, distinguish between the different stages of infection and monitor treatment success.

Funding information We would like to acknowledge the funding assistance from the Malaysian Ministry of Education through the Higher Institution Centre of Excellence Program (HICoE) [Grant no. 311/CIPPM/4401005].

References

1. Stanley SL (2003) Amoebiasis. Lancet 361:1025–1034
2. Collier P (2007) The bottom billion: why the poorest countries are failing and what can be done about it. Oxford University Press, United Kingdom
3. Fotedar R, Stark D, Beebe N, Marriott D, Ellis J, Harkness J (2007a) Laboratory diagnostic techniques for *Entamoeba* species. Clin Microbiol Rev 20:511–532
4. Zlobl TL (2001) Amebiasis. Prim Care Update Ob Gyns 8:65–68
5. Stanley SL (2006) Vaccines for amoebiasis: barriers and opportunities. Parasitology 133:S81–S86
6. Hung CC, Ji DD, Sun HY, Lee YT, Hsu SY, Chang SY, Wu CH, Chan YH, Hsiao CF, Liu WC, Colebunders R (2008) Increased risk for *Entamoeba histolytica* infection and invasive amebiasis in HIV seropositive men who have sex with men in Taiwan. PLoS Negl Trop Dis 2:e175
7. Rivero LR, Fernández FAN, Robertson LJ (2008) Cuban parasitology in review: a revolutionary triumph. Trends Parasitol 24: 440–448
8. Gathiram V, Jackson TF (1987) A longitudinal study of asymptomatic carriers of pathogenic zymodemes of *Entamoeba histolytica*. S Afr Med J 72:669–672
9. Caballero-Salcedo A, Viveros-Rogel M, Salvatierra B, Tapia-Conyer R, Sepulveda-Amor J, Gutierrez G, Ortiz-Ortiz L (1994) Seroepidemiology of amebiasis in Mexico. Am J Trop Med Hyg 50:412–419
10. Hughes MA, Petri WA (2000) Amebic liver abscess. Infect Dis Clin N Am 14:565–582
11. Garcia LS, Shimizu RY (1998) Evaluation of intestinal protozoan morphology in human fecal specimens preserved in EcoFix: comparison of Wheatley's trichrome stain and EcoStain. Clin Microbiol 36:1974–1976
12. Gonzalez-Ruiz A, Haque R, Aguirre A, Castanon G, Hall A, Guhl F, Ruiz-Palacios G, Miles MA, Warhurst DC (1994) Value of microscopy in the diagnosis of dysentery associated with invasive *Entamoeba histolytica*. J Clin Pathol 47:236–239
13. Haque R, Neville LM, Hahn P, Petri WA (1995) Rapid diagnosis of *Entamoeba* infection by using *Entamoeba* and *Entamoeba histolytica* stool antigen detection kits. J Clin Microbiol 33:2558–2561
14. World Health Organization (WHO) (1997) UNESCO report of a consultation of experts on amoebiasis. Wkly Epidemiol Rec 72:97–99
15. Tanyuksel M, Petri WA (2003) Laboratory diagnosis of amebiasis. Clin Microbiol Rev 16:713–729
16. Haque R, Petri WA (2006) Diagnosis of amebiasis in Bangladesh. Arch Med Res 37:272–275
17. Liang SY, Chan YH, Hsia KT, Lee JL, Kuo MC, Hwa KY, Chan CW, Chiang TY, Chen JS, Wu FT, Ji DD (2009) Development of loop-mediated isothermal amplification assay for detection of *Entamoeba histolytica*. J Clin Microbiol 47:1892–1895
18. Clark CG, Diamond LS (2002) Methods for cultivation of luminal parasitic protists of clinical importance. Clin Microbiol Rev 15:329–341

19. Freedman L, Maddison SE, Elsdon-Dew B (1958) Monoxenic cultures of *Entamoeba histolytica* derived from human liver abscesses. Afr J Med Med Sci 23:9–12
20. Wang LT, Jen G, Cross JH (1973) Establishment of *Entamoeba histolytica* from liver abscess in monoxenic cultures with hemoflagellates. Am J Trop Med Hyg 22:30–32
21. Sargeaunt PG, Jackson TF, Wiffen S, Bhojnani R, Williams JE, Felmingham D, Goldmeir D, Allason-Jones E, Mindel A, Phillips E (1987) The reliability of *Entamoeba histolytica* zymodemes in clinical laboratory diagnosis. Arch Invst Med 18:69–75
22. Razmjou E, Haghighi A, Rezaian M, Kobayashi S, Nozaki T (2006) Genetic diversity of glucose phosphate isomerase from *Entamoeba histolytica*. Parasitol Int 55:307–311
23. Jackson TF, Suparsad S (1996) Zymodeme stability of *Entamoeba histolytica* and *E. dispar*. Arc Med Res 28:304–305
24. Strachan WD, Spice WM, Chiodini PL, Moody AH, Ackers JP (1988) Immunological differentiation of pathogenic and non-pathogenic isolates of *Entamoeba histolytica*. Lancet 331:561–563
25. Haque R, Kress K, Wood S, Jackson TF, Lyerly D, Wilkins T, Petri WA (1993) Diagnosis of pathogenic *Entamoeba histolytica* infection using a stool ELISA based on monoclonal antibodies to the galactose-specific adhesin. J Infect Dis 167:247–249
26. Haque R, Faruque ASG, Hahn P, Lyerly DM, Petri WA (1997) *Entamoeba histolytica* and *Entamoeba dispar* infection in children in Bangladesh. J Infec Dis 175:734–736
27. Haque R, Ali IKM, Akther S, Petri WA (1998a) Comparison of PCR, isoenzyme analysis, and antigen detection for diagnosis of *Entamoeba histolytica* infection. J Clin Microbiol 36:449–452
28. Haque R, Ali IKM, Clark CG, Petri WA (1998b) A case report of *Entamoeba moshkovskii* infection in a Bangladeshi child. Parasitol Int 47:201–202
29. Gatti S, Swierczynski G, Robinson F, Anselmi M, Corrales J, Moreira J, Montalvo G, Bruno A, Maserati R, Bisoffi Z, Scaglia M (2002) Amebic infections due to the *Entamoeba histolytica-Entamoeba dispar* complex: a study of the incidence in a remote rural area of Ecuador. Am J Trop Med Hyg 67:123–127
30. Roy S, Kabir M, Mondal D, Ali IKM, Petri WA, Haque R (2005) Real-time-PCR assay for diagnosis of *Entamoeba histolytica* infection. J Clin Microbiol 43:2168–2172
31. 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 J Med Microbiol 296:397–403
32. Mohanty S, Sharma N, Deb M (2014) Microscopy versus enzyme linked immunosorbent assay test for detection of *Entamoeba histolytica* infection in stool samples. Trop Parasitol 4:136
33. Al-Basheer NM, Majeed IA, Jawad HM (2014) Evaluation of antigen-based enzyme immuno-assay in reference to direct microscopy for the diagnosis of *Entamoeba histolytica* in stool samples. Al-Taqani 27:E54–E60
34. Akhtar T, Khan AG, Ahmed I, Nazli R, Haider J (2016) Prevalence of amoebiasis in a model research community and its confirmation using stool antigen elisa for *Entamoeba histolytica*. Pak J Pharm Sci 29:1587–1590
35. Ong SJ, Cheng MY, Liu KH, Horng CB (1996) Use of the ProSpecT® microplate enzyme immunoassay for the detection of pathogenic and non-pathogenic *Entamoeba histolytica* in faecal specimens. Trans R Soc Trop Med Hyg 90:248–249
36. Pillai DR, Kain KC (1999) Immunochromatographic strip-based detection of *Entamoeba histolytica-E. dispar* and *Giardia lamblia* coproantigen. J Clin Microbiol 37:3017–3019
37. Hira PR, Iqbal J, Al-Ali FAIZA, Philip R, Grover S, D'Almeida E, Al-Eneizi AA (2001) Invasive amebiasis: challenges in diagnosis in a nonendemic country (Kuwait). Am J Trop Med Hyg 65:341–345
38. van Hal SJ, Stark DJ, Fotedar R, Marriott D (2007) Amoebiasis: current status in Australia. Med J Aust 186:412
39. van Doorn HR, Hofwegen H, Koelewijn R, Gilis H, Peek R, Wetsteyn JC, van Genderen PJ, Vervoort T, van Gool T (2005) Use of rapid dipstick and latex agglutination tests and enzyme-linked immunosorbent assay for serodiagnosis of amebic liver abscess, amebic colitis, and *Entamoeba histolytica* cyst passage. J Clin Microbiol 43:4801–4806
40. Shenai BR, Komalam BL, Arvind AS, Krishnaswamy PR, Rao PV (1996) Recombinant antigen-based avidin-biotin microtiter enzyme-linked immunosorbent assay for serodiagnosis of invasive amebiasis. J Clin Microbiol 34:828–833
41. Uslu H, Aktas O, Uyanik MH (2016) Comparison of various methods in the diagnosis of *Entamoeba histolytica* in stool and serum specimens. Eur J Med 48:124
42. Knappik M, Börner U, Jelinek T (2005) Sensitivity and specificity of a new commercial enzyme-linked immunoassay kit for detecting *Entamoeba histolytica* IgG antibodies in serum samples. Eur J Clin Microbiol Infect Dis 24:701–703
43. Dhanalakshmi S, Meenachi C, Parija SC (2016) Indirect haemagglutination test in comparison with ELISA for detection of antibodies against invasive amoebiasis. J Clin Diagn Res 10: DC05–DC08
44. Dhanalakshmi S, Parija SC (2016) Seroprevalence of *Entamoeba histolytica* from a tertiary care hospital, South India. Trop Parasitol 6:78
45. Garcia LS, Shimizu RY, Bernard CN (2000) Detection of *Giardia lamblia*, *Entamoeba histolytica*/*Entamoeba dispar*, and *Cryptosporidium parvum* antigens in human fecal specimens using the triage parasite panel enzyme immunoassay. J Clin Microbiol 38:3337–3340
46. Sharp SE, Suarez CA, Duran Y, Poppiti RJ (2001) Evaluation of the triage micro parasite panel for detection of *Giardia lamblia*, *Entamoeba histolytica*/*Entamoeba dispar*, and *Cryptosporidium parvum* in patient stool specimens. J Clin Microbiol 39:332–334
47. Gaafar MR (2011) Evaluation of enzyme immunoassay techniques for diagnosis of the most common intestinal protozoa in fecal samples. Int J Infect Dis 15(8):e541–e544
48. Van den Bossche D, Cnops L, Verschueren J, Van Esbroeck M (2015) Comparison of four rapid diagnostic tests, ELISA, microscopy and PCR for the detection of *Giardia lamblia*, *Cryptosporidium* spp. and *Entamoeba histolytica* in feces. J Microbiol Methods 110:78–84
49. Goñi P, Martín B, Villacampa M, Garcia A, Seral C, Castillo FJ, Clavel A (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 31:2077–2082
50. Formenti F, Perandin F, Bonafini S, Degani M, Bisoffi Z (2015) Evaluation of the new ImmunoCard STAT!® CGE test for the diagnosis of Amebiasis. Bull Soc Pathol Exot 108:171–174
51. Ibrahim SS, El-Matarawy OM, Ghieth MA, Sarea EYA, El-Badry AA (2015) Copro prevalence and estimated risk of *Entamoeba histolytica* in diarrheic patients at Beni-Suef, Egypt. World J Microbiol Biotechnol 31:385–390
52. Korpe PS, Stott BR, Nazib F, Kabir M, Haque R, Herbein JF, Petri WA (2012) Evaluation of a rapid point-of-care fecal antigen detection test for *Entamoeba histolytica*. Am J Trop Med Hyg 86:980–981
53. Verkerke HP, Hanbury B, Siddique A, Samie A, Haque R, Herbein J, Petri WA (2015) Multisite clinical evaluation of a rapid test for *Entamoeba histolytica* in stool. J Clin Microbiol 53:493–497
54. Saidin S, Yunus MH, Othman N, Lim YAL, Mohamed Z, Zakaria NZ, Noordin R (2017) Development and initial evaluation of a

lateral flow dipstick test for antigen detection of *Entamoeba histolytica* in stool sample. Pathog Glob Health 111:128–136
55. Gonin P, Trudel L (2003) Detection and differentiation of *Entamoeba histolytica* and *Entamoeba dispar* isolates in clinical samples by PCR and enzyme-linked immunosorbent assay. J Clin Microbiol 41:237–241
56. Haque R, Mollah NU, Ali IKM, Alam K, Eubanks A, Lyerly D, Petri WA (2000) Diagnosis of amebic liver abscess and intestinal infection with the TechLab *Entamoeba histolytica* II antigen detection and antibody tests. J Clin Microbiol 38:3235–3239
57. Stark D, van Hal SJ, Matthews G, Harkness J, Marriott D (2008) Invasive amebiasis in men who have sex with men, Australia. Emerg Infect Dis 14:1141
58. Liang SY, Hsia KT, Chan YH, Fan CK, Jiang DDS, Landt O, Ji DD (2010) Evaluation of a new single-tube multiprobe real-time PCR for diagnosis of *Entamoeba histolytica* and *Entamoeba dispar*. J Parasitol 96:793–797
59. Mirelman D, Nuchamowitz Y, Stolarsky T (1997) Comparison of use of enzyme-linked immunosorbent assay-based kits and PCR amplification of rRNA genes for simultaneous detection of *Entamoeba histolytica* and *E. dispar*. J Clin Microbiol 35:2405–2407
60. Petri WA, Singh U (1999) Diagnosis and management of amebiasis. Clin Infect Dis 29:1117–1125
61. Abd-Alla MD, Jackson TF, Reddy S, Ravdin JI (2000) Diagnosis of invasive amebiasis by enzyme-linked immunosorbent assay of saliva to detect amebic lectin antigen and anti-lectin immunoglobulin G antibodies. J Clin Microbiol 38:2344–2347
62. Gomes TDS, Garcia MC, Souza Cunha FD, Werneck de Macedo H, Peralta JM Peralta RHS (2014) Differential diagnosis of *Entamoeba* spp. in clinical stool samples using SYBR Green real-time polymerase chain reaction. Sci World J. https://doi.org/10.1155/2014/645084
63. Haque R, Kabir M, Noor Z, Rahman SM, Mondal D, Alam F, Rahman I, Al Mahmood A, Ahmed N, Petri WA (2010) Diagnosis of amebic liver abscess and amebic colitis by detection of *Entamoeba histolytica* DNA in blood, urine, and saliva by a real-time PCR assay. J Clin Microbiol 48:2798–2801
64. Khairnar K, Parija SC, Palaniappan R (2007) Diagnosis of intestinal amoebiasis by using nested polymerase chain reaction-restriction fragment length polymorphism assay. J Gastroenterol 42:631–640
65. Calderaro A, Gorrini C, Bommezzadri S, Piccolo G, Dettori G, Chezzi C (2006) *Entamoeba histolytica* and *Entamoeba dispar*: comparison of two PCR assays for diagnosis in a non-endemic setting. Trans R Soc Trop Med Hyg 100:450–457
66. Blessmann J, Buss H, Nu PA, Dinh BT, Ngo QT, Le Van A, Alla MD, Jackson TF, Ravdin JI, Tannich E (2002) Real-time PCR for detection and differentiation of *Entamoeba histolytica* and *Entamoeba dispar* in fecal samples. J Clin Microbiol 40:4413–4417
67. Shah PH, MacFarlane RC, Bhattacharya D, Matese JC, Demeter J, Stroup SE Singh U (2005) Comparative genomic hybridizations of *Entamoeba* strains reveal unique genetic fingerprints that correlate with virulence. Eukaryot Cell 4:504–515
68. Zaman S, Khoo J, Ng SW, Ahmed R, Khan MA, Hussain R, Zaman V (2000) Direct amplification of *Entamoeba histolytica* DNA from amoebic liver abscess pus using polymerase chain reaction. J Parasitol Res 86:724–728
69. Zindrou S, Orozco E, Linder E, Téllez A, Björkman A (2001) Specific detection of *Entamoeba histolytica* DNA by hemolysin gene targeted PCR. Acta Trop 78:117–125
70. Hamzah Z, Petmitr S, Mungthin M, Leelayoova S, Chavalitshewinkoon-Petmitr P (2006) Differential detection of *Entamoeba histolytica*, *Entamoeba dispar*, and *Entamoeba moshkovskii* by a single-round PCR assay. J Clin Microbiol 44:3196–3200
71. Sri-Hidajati BS, Basuki S, Pusarawati S, Kusmartisnawati K, Rossyanti L, Sulistyowati SW, Kartikasari DP, Arwati H, Tantula I, Darma A, Handajani R (2018) Comparison of multiplex single round PCR and microscopy in diagnosis of amoebiasis. Afr J Infect Dis 12:120–126
72. Ali IK, Hossain MB, Roy S, Ayeh-Kumi PF, Petri WA Jr, Haque R, Clark CG (2003) *Entamoeba moshkovskii* infections in children, Bangladesh. Emerg Infect Dis 9:580–584
73. Parija SC, Khairnar K (2005) *Entamoeba moshkovskii* and *Entamoeba dispar*-associated infections in Pondicherry, India. J Health Popul Nutr 23:292–295
74. Ngui R, Angal L, Fakhrurrazi SA, Lian YLA, Ling LY, Ibrahim J, Mahmud R (2012) Differentiating *Entamoeba histolytica*, *Entamoeba dispar* and *Entamoeba moshkovskii* using nested polymerase chain reaction (PCR) in rural communities in Malaysia. Parasit Vectors 5:187
75. Lau YL, Anthony C, Fakhrurrazi SA, Ibrahim J, Ithoi I, Mahmud R (2013) Real-time PCR assay in differentiating *Entamoeba histolytica*, *Entamoeba dispar*, and *Entamoeba moshkovskii* infections in Orang Asli settlements in Malaysia. Parasit Vectors 6:250
76. López-López P, Martínez-López MC, Boldo-León XM, Hernández-Díaz Y, González-Castro TB, Tovilla-Zárate CA, Luna-Arias JP (2017) Detection and differentiation of *Entamoeba histolytica* and *Entamoeba dispar* in clinical samples through PCR-denaturing gradient gel electrophoresis. Braz J Med Biol Res. https://doi.org/10.1590/1414-431x20175997
77. Fallah E, Shahbazi A, Yazdanjoii M, Rahimi-Esboei B (2014) Differential detection of *Entamoeba histolytica* from *Entamoeba dispar* by parasitological and nested multiplex polymerase chain reaction methods. J Anal Res Clin Med 2:27–32
78. Dawah IS, Inabo HI, Abdullahi IO, Machido AD (2016) Differentiation of *Entamoeba histolytica*, *Entamoeba dispar* and *Entamoeba moshkovskii* from diarrhoeic stools using polymerase chain reaction in Kaduna, Nigeria. Int J Biomed Sci 5:61–66
79. Al-Areeqi MA, Sady H, Al-Mekhlafi HM, Anuar TS, Al-Adhroey AH, Atroosh WM et al (2017) First molecular epidemiology of *Entamoeba histolytica*, E. *dispar* and E. *moshkovskii* infections in Yemen: different species-specific associated risk factors. Tropical Med Int Health 22:493–504
80. Qvarnstrom Y, James C, Xayavong M, Holloway BP, Visvesvara GS, Sriram R, da Silva AJ (2005) Comparison of real-time PCR protocols for differential laboratory diagnosis of amebiasis. J Clin Microbiol 43:5491–5497
81. Verweij JJ, Blangé RA, Templeton K, Schinkel J, Brienen EA, van Rooyen MA, van Lieshout L, Polderman AM (2004) Simultaneous detection of *Entamoeba histolytica*, *Giardia lamblia*, and *Cryptosporidium parvum* in fecal samples by using multiplex real-time PCR. J Clin Microbiol 42:1220–1223
82. Roshdy MH, El-Kader NMA, Ali-Tammam M, Fuentes I, Mohamed MM, El-Sheikh NA, Rubio JM (2017) Molecular diagnosis of *Entamoeba* spp. versus microscopy in the Great Cairo. Acta Parasitol 62:188–191
83. Nazeer JT, Khalifa KES, von Thien H, El-Sibaei MM, Abdel-Hamid MY, Tawfik RAS, Tannich E (2013) Use of multiplex real-time PCR for detection of common diarrhea causing protozoan parasites in Egypt. Parasitol Res 112:595–601
84. Ahmad N, Khan M, Hoque MI, Haque R, Mondol D (2007) Detection of *Entamoeba histolytica* DNA from liver abscess aspirate using polymerase chain reaction (PCR): a diagnostic tool for amoebic liver abscess. Bangladesh Med Res Counc Bull 33:13-20
85. Aguayo-Patrón S, Castillo-Fimbres R, Quihui-Cota L, de la Barca AMC (2017) Use of real-time polymerase chain reaction to

identify *Entamoeba histolytica* in schoolchildren from northwest Mexico. J Infect Dev Ctries 11:800–805
86. Hamzah Z, Petmitr S, Mungthin M, Leelayoova S, Chavalitshewinkoon-Petmitr P (2010) Development of multiplex real-time polymerase chain reaction for detection of *Entamoeba histolytica*, *Entamoeba dispar*, and *Entamoeba moshkovskii* in clinical specimens. Am J Trop Med Hyg 83:909–913
87. Singh P, Mirdha BR, Ahuja V, Singh S (2013) Loop-mediated isothermal amplification (LAMP) assay for rapid detection of *Entamoeba histolytica* in amoebic liver abscess. World J Microbiol Biotechnol 29:27–32
88. Rivera WL, Ong VA (2013) Development of loop-mediated isothermal amplification for rapid detection of *Entamoeba histolytica*. Asian Pac J Trop Med 6:457–461
89. Mwendwa F, Mbae CK, Kinyua J, Mulinge E, Mburugu GN, Njiru ZK (2017) Stem loop-mediated isothermal amplification test: comparative analysis with classical LAMP and PCR in detection of *Entamoeba histolytica* in Kenya. BMC Res Notes 10:142
90. Foo PC, Chan YY, Mohamed M, Wong WK, Najian AN, Lim BH (2017) Development of a thermostabilised triplex LAMP assay with dry-reagent four target lateral flow dipstick for detection of *Entamoeba histolytica* and non-pathogenic *Entamoeba* spp. Anal Chim Acta 966:71–80
91. Freitas MAR, Vianna EN, Martins AS, Silva EF, Pesquero JL, Gomes MA (2004) A single step duplex PCR to distinguish *Entamoeba histolytica* from *Entamoeba dispar*. Parasitology 128:625–628
92. Núñez YO, Fernández MA, Torres-Núñez D, Silva JA, Montano I, Maestre JL, Fonte L (2001) Multiplex polymerase chain reaction amplification and differentiation of *Entamoeba histolytica* and *Entamoeba dispar* DNA from stool samples. Am J Trop Med Hyg 64:93–297
93. Bhattacharya S, Som I, Bhattacharya A (1998) The ribosomal DNA plasmids of *Entamoeba*. Parasitol Today 14:181–185
94. Cruz-Reyes JA, Spice WM, Rehman T, Gisborne E, Ackers JP (1992) Ribosomal DNA sequences in the differentiation of pathogenic and non-pathogenic isolates of *Entamoeba histolytica*. Parasitology 104:239–246
95. Nath J, Ghosh SK, Singha B, Paul J (2015) Molecular epidemiology of amoebiasis: a cross-sectional study among North East Indian population. PLoS Neg Trop Dis 9:e0004225
96. Khairnar K, Parija SC (2007) A novel nested multiplex polymerase chain reaction (PCR) assay for differential detection of *Entamoeba histolytica*, *E. moshkovskii* and *E. dispar* DNA in stool samples. BMC Microbiol 7:1
97. ElBakri A, Samie A, Ezzedine S, Odeh RA (2013) Differential detection of *Entamoeba histolytica*, *Entamoeba dispar* and *Entamoeba moshkovskii* in fecal samples by nested PCR in the United Arab Emirates (UAE). Acta Parasitol 58:185–190
98. Verweij JJ, Laeijendecker D, Brienen EA, van Lieshout L, Polderman AM (2003) Detection and identification of *Entamoeba* species in stool samples by a reverse line hybridization assay. J Clin Microbiol 41:5041–5045
99. Binnicker MJ (2015) Multiplex molecular panels for diagnosis of gastrointestinal infection: performance, result interpretation, and cost-effectiveness. J Clin Microbiol 53:3723–3728
100. Zboromyrska Y, Vila J (2016) Advanced PCR-based molecular diagnosis of gastrointestinal infections: challenges and opportunities. Expert Rev Mol Diagn 16:631–640
101. Shakir JM (2015) Evaluation of multiplex real-time PCR for detection of three diarrhea causing intestinal protozoa. Med Sci 5:783–786
102. Weitzel T, Cabrera J, Rosas R, Noriega LM, Schiappacasse G, Vollrath V, Porte L (2017) Enteric multiplex PCR panels: a new diagnostic tool for amoebic liver abscess? New Microbes New Infect 18:50–53
103. Navidad JF, Griswold DJ, Gradus MS, Bhattacharyya S (2013) Evaluation of Luminex xTAG® gastrointestinal pathogen analyte specific reagents for high-throughput, simultaneous detection of bacteria, viruses, and parasites of clinical and public health importance. J Clin Microbiol 51:3018–3024
104. Morio F, Valot S, Laude A, Desoubeaux G, Argy N, Nourrisson C, Pomares C, Machouart M, Le Govic Y, Dalle F, Botterel F (2018) Evaluation of a new multiplex PCR assay (ParaGENIE G-Amoeba real-time PCR kit) targeting Giardia intestinalis, *Entamoeba histolytica* and *Entamoeba dispar/Entamoeba moshkovskii* from stool specimens: evidence for the limited performances of microscopy-based approach for amoeba species identification. Clin Microbiol Infect. https://doi.org/10.1016/j.cmi.2018.02.007
105. Laude A, Valot S, Desoubeaux G, Argy N, Nourrisson C, Pomares C, Machouart M, Le Govic Y, Dalle F, Botterel F, Bourgeois N (2016) Is real-time PCR-based diagnosis similar in performance to routine parasitological examination for the identification of *Giardia intestinalis*, *Cryptosporidium parvum*/*Cryptosporidium hominis* and *Entamoeba histolytica* from stool samples? Evaluation of a new commercial multiplex PCR assay and literature review. Clin Microbiol Infect. https://doi.org/10.1016/j.cmi.2015.10.019
106. Parija SC, Mandal J, Ponnambath DK (2014) Laboratory methods of identification of *Entamoeba histolytica* and its differentiation from look-alike *Entamoeba* spp. Trop Parasitol 4:90
107. Notomi T, Okayama H, Masubuchi H, Yonekawa T, Watanabe K, Amino N, Hase T (2000) Loop-mediated isothermal amplification of DNA. Nucleic Acids Res 28:e63–e63
108. Fernández-Soto P, Arahuetes JG, Hernández AS, Abán JL, Santiago BV, Muro A (2014) A loop-mediated isothermal amplification (LAMP) assay for early detection of *Schistosoma mansoni* in stool samples: a diagnostic approach in a murine model. PLoS Negl Trop Dis 8:e3126
109. Banoo S, Bell D, Bossuyt P, Herring A, Mabey D, Poole F, Smith PG, Sriram N, Wongsrichanalai C, Linke R, O'brien R (2008) Evaluation of diagnostic tests for infectious diseases: general principles. Nat Rev Microbiol 8:S16–S28
110. Chin CD, Chin SY, Laksanasopin T, Sia SK (2013) Low-cost micron- devices for point-of-care testing. In: Issadore D, Westervelt RM (eds) Point-of-care diagnostics on a Chip. Springer, Berlin Heidelberg, pp 3–21
111. Peeling RW, Mabey D (2010) Point-of-care tests for diagnosing infections in the developing world. Clin Microbiol Infect 16:1062–1069
112. Leiva B, Lebbad M, Winiecka-Krusnell J, Altamirano I, Tellez A, Linder E (2006) Overdiagnosis of *Entamoeba histolytica* and *Entamoeba dispar* in Nicaragua: a microscopic, triage parasite panel and PCR study. Arch Med Res 37:529–534
113. Leo M, Haque R, Kabir M, Roy S, Lahlou RM, Mondal D, Tannich E, Petri WA (2006) Evaluation of *Entamoeba histolytica* antigen and antibody point-of-care tests for the rapid diagnosis of amebiasis. J Clin Microbiol 44:4569–4571
114. Mukhopadhyay M, Saha AK, Sarkar A, Mukherjee S (2010) Amoebic liver abscess: presentation and complications. Indian J Surg 72:37–41
115. Kasper DL, Braunwald E, Fauci AS, Hauser SL, Longo DL, Jameson JL (2005) Harrison's principles of internal medicine. McGraw-Hill companies Inc, USA
116. Katzenstein D, Rickerson V, Braude A (1982) New concepts of amebic liver abscess derived from hepatic imaging, serodiagnosis, and hepatic enzymes in 67 consecutive cases in San Diego. Medicine 61:237–246
117. Mathur S, Gehlot RS, Mohta A, Bhargava N (2002) Clinical profile of amoebic liver abscess. J Indian Acad Clin Med 3:367–373

118. Salles JM, Moraes LA, Salles MC (2003) Hepatic amebiasis. Braz J Infect Dis 7:96–110
119. Othman N, Mohamed Z, Verweij JJ, Huat LB, Olivos-García A, Yeng C, Noordin R (2010) Application of real-time polymerase chain reaction in detection of *Entamoeba histolytica* in pus aspirates of liver abscess patients. Foodborne Pathog Dis 7:637–641
120. Sifri CD, Madoff LC (2010) Infectious disease of the liver and biliary system. In: Mandell GL, Benett JE, Dolin R (eds) Principles and practice of infectious disease. Churchill Livingstone Elsevier, Philadelphia, pp 1035–1044
121. Parija SC, Khairnar K (2007) Detection of excretory *Entamoeba histolytica* DNA in the urine, and detection of *E. histolytica* DNA and lectin antigen in the liver abscess pus for the diagnosis of amoebic liver abscess. BMC Microbiol 7:41
122. Bancroft JD, Gamble M (2002) Theory and practice of histological techniaques, 5th edn. Churchill Livingstone, China
123. Rigothier MC, Khun H, Tavares P, Cardona A, Huerre M, Guillen N (2002) Fate of *Entamoeba histolytica* during establishment of amoebic liver abscess analyzed by quantitative radioimaging and histology. Infect Immun 70:3208–3215
124. Ning TZ, Kin WW, Mustafa S, Ahmed A, Noordin R, Cheong TG, Huat LB (2012) Detection of *Entamoeba histolytica* in experimentally induced amoebic liver abscess: comparison of three staining methods. Asian Pac J Trop Biomed 2:61–65
125. Zengzhu G, Bracha R, Nuchamowitz Y, Cheng W, Mirelman D (1999) Analysis by enzyme-linked immunosorbent assay and PCR of human liver abscess aspirates from patients in China for *Entamoeba histolytica*. J Clin Microbiol 37:3034–3036
126. Karki BM, Parija SC (1999) Co-agglutination test for the detection of circulating antigen in amebic liver abscess. Am J Trop Med Hyg 60:498–501
127. Ravdin JI, Jackson TF, Petri WA Jr, Murphy CF, Ungar BL, Gathiram V, Skilogiannis J, Simjee AE (1990) Association of serum antibodies to adherence lectin with invasive amebiasis and asymptomatic infection with pathogenic *Entamoeba histolytica*. J Infect Dis 162:768–772
128. Hung CC, Chen PJ, Hsieh SM, Wong JM, Fang CT, Chang SC, Chen MY (1999) Invasive amoebiasis: an emerging parasitic disease in patients infected with HIV in an area endemic for amoebic infection. Aids 13:2421–2428
129. del Carmen Sánchez-Guillén M, Velázquez-Rojas M, Salgado-Rosas H, Torres-Rasgado E, Pérez-Fuentes R, Martínez-Munguía J, Talamás-Rohana P (2000) Seroprevalence of anti-*Entamoeba histolytica* antibodies by IHA and ELISA assays in blood donors from Puebla, Mexico. Arch Med Res 31:S53–S54
130. Cross JH, Clarke MD, Cole WC, Lien JC, Partono F, Joesoef A, Oemijati S (1976) Parasitic infections in humans in West Kalimantan (Borneo), Indonesia. Trop Geogr Med 28:121–130
131. Mohamed Z, Bachok NA, Hasan H (2009) Analysis of indirect hemagglutination assay results among patients with amoebic liver abscess. Int Med J 16:195–199
132. Garcia LS, Bruckner DA, Brewer TC, Shimizu RY (1982) Comparison of indirect fluorescent-antibody amoebic serology with counterimmunoelectrophoresis and indirect hemagglutination amoebic serologies. J Clin Microbiol 15:603-605
133. Shamsuzzaman SM, Haque R, Ruhul Hasin SK, Hashiguchi Y (2000) Evaluation of indirect fluorescent antibody test and enzyme-linked immunosorbent assay for diagnosis of hepatic amebiasis in Bangladesh. J Parasitol 86:611-615
134. Patterson M, Schoppe LE (1982) Presentation of amoebiasis. Med Clin North Am 66:689–705
135. Yang J, Kennedy MT (1979) Evaluation of enzyme-linked immunosorbent assay for the serodiagnosis of amebiasis. J Clin Microbiol 10:778-785
136. Robert R, Mahaza C, Bernard C, Buffard C, Senet JM (1990) Evaluation of a new bicolored latex agglutination test for immunological diagnosis of hepatic amoebiasis. J Clin Microbiol 28:1422–1424
137. Wong WK, Tan ZN, Othman N, Lim BH, Mohamed Z, Garcia AO, Noordin R (2011) Analysis of *Entamoeba histolytica*-excretory-secretory antigen and identification of a new potential diagnostic marker. Clin Vaccine Immunol 18:1913–1917
138. Lotter H, Mannweiler E, Schreiber M, Tannich E (1992) Sensitive and specific serodiagnosis of invasive amebiasis by using a recombinant surface protein of pathogenic E*ntamoeba histolytica*. J Clin Microbiol 30:3163–3167
139. Weinke T, Scherer W, Neuber U, Trautmann M (1989) Clinical features and management of amebic liver abscess. Experience from 29 patients. Klin Wochenschr 67:415–420
140. Saidin S, Yunus MH, Zakaria ND, Razak KA, Huat LB, Othman N, Noordin R (2014) Production of recombinant *Entamoeba histolytica* pyruvate phosphate dikinase and its application in a lateral flow dipstick test for amoebic liver abscess. BMC Infect Dis 14:182
141. Myung K, Burch D, Jackson TF, Stanley SL Jr (1991) Serodiagnosis of invasive amebiasis using a recombinant *Entamoeba histolytica* antigen-based ELISA. Arch Med Res 23:285–288
142. Tachibana H, Cheng XJ, Masuda G, Horiki N, Takeuchi T (2004) Evaluation of recombinant fragments of *Entamoeba histolytica* Gal/GalNAc lectin intermediate subunit for serodiagnosis of amebiasis. J Clin Microbiol 42:1069–1074
143. Stanley SL (1997) Progress towards development of a vaccine for amebiasis. Clin Microbiol Rev 10:637–649
144. Kimura A, Hara Y, Kimoto T, Okuno Y, Minekawa Y, Nakabayashi T (1996) Cloning and expression of a putative alcohol dehydrogenase gene of *Entamoeba histolytica* and its application to immunological examination. Clin Diagn Lab Immunol 3:270–274
145. Ning TZ, Kin WW, Noordin R, Cun ST, Chong FP, Mohamed Z, Olivos-Garcia A, Huat LB (2013) Evaluation of *Entamoeba histolytica* recombinant phosphoglucomutase protein for serodiagnosis of amoebic liver abscess. BMC Infect Dis 13:144
146. Khan U, Mirdha BR, Samantaray JC, Sharma MP (2006) Detection of *Entamoeba histolytica* using polymerase chain reaction in pus samples from amebic liver abscess. Indian J Gastroenterol 25:55
147. Tachibana H, Kobayashi S, Okuzawa E, Masuda G (1992) Detection of pathogenic *Entamoeba histolytica* DNA in liver abscess fluid by polymerase chain reaction. Int J Parasitol 22:1193–1196
148. Morshed M, Cherian SS, Lo T, Lee MK, Wong Q, Hoang L (2017) Superiority of PCR against microscopy for diagnosing *Entamoeba histolytica* in liver abscess samples. Can J Infect Dis Med Microbiol 2:1–3
149. Pritt BS, Clark CG (2008) Amebiasis. Mayo Clin Proc 83:1154–1160
150. Dhawan VK (2008) Current diagnosis and treatment of amebiasis. US Infect Dis 4:59–61
151. Tachibana H, Kakino A, Kazama M, Feng M, Asai S, Umezawa K, Nozaki T, Makiuchi T, Kamada T, Watanabe H, Horiki N (2018) Development of a sensitive immunochromatographic kit using fluorescent silica nanoparticles for rapid serodiagnosis of amebiasis. Parasitology 9:1–6
152. Shastry BS (2002) SNP alleles in human disease and evolution. J Hum Genet 47:561
153. Luna-Nácar M, Navarrete-Perea J, Moguel B, Bobes RJ, Laclette JP, Carrero JC (2016) Proteomic study of *Entamoeba histolytica* trophozoites, cysts, and cyst-like structures. PLoS One 11: e0156018
154. Ujang JA, Kwan SH, Ismail MN, Lim BH, Noordin R, Othman N (2016) Proteome analysis of excretory-secretory proteins

of *Entamoeba histolytica* HM1: IMSS via LC–ESI–MS/MS and LC–MALDI–TOF/TOF. Clin Proteomics 13:33

155. Perdomo D, Aït-Ammar N, Syan S, Sachse M, Jhingan GD, Guillén N (2015) Cellular and proteomics analysis of the endomembrane system from the unicellular *Entamoeba histolytica*. J Proteome 112:125–140
156. Biller L, Matthiesen J, Kuehne V, Lotter H, Handal G, Nozaki T, Saito-Nakano Y, Schuemann M, Roeder T, Tannich E, Krause E (2013) The cell surface proteome of *Entamoeba histolytica*. Mol Cell Proteomics. https://doi.org/10.1074/mcp.M113.031393
157. Okada M, Huston CD, Oue M, Mann BJ, Petri WA Jr, Kita K, Nozaki T (2006) Kinetics and strain variation of phagosome proteins of *Entamoeba histolytica* by proteomic analysis. Mol Biochem Parasitol 145:171–183
158. Leitsch D, Radauer C, Paschinger K, Wilson IB, Breiteneder H, Scheiner O, Duchêne M (2005) *Entamoeba histolytica*: analysis of the trophozoite proteome by two-dimensional polyacrylamide gel electrophoresis. Exp Parasitol 110:191–195
159. Davis PH, Chen M, Zhang X, Clark CG, Townsend RR, Stanley SL Jr (2009) Proteomic comparison of *Entamoeba histolytica* and *Entamoeba dispar* and the role of *E. histolytica* alcohol dehydrogenase 3 in virulence. PLoS Negl Trop Dis 3:e415
160. Davis PH, ZhangX, Guo J, Townsend RR, Samuel L. Stanley, (2006) Comparative proteomic analysis of two *Entamoeba histolytica* strains with different virulence phenotypes identifies peroxiredoxin as an important component of amoebic virulence. Mol Microbiol 61:1523-1532