Diagnostic performances of antigen detection compared to conventional and nucleic acid detection of *Entamoeba histolytica* in a non-endemic setting

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**SUMMARY**

This study evaluated the immunochromatographic (IC) assay “TECHLAB® *E. HISTOLYTICA QUIK CHEK™*” analysing 36 faecal samples and 7 cultured strains. This assay was compared to the methods performed in our laboratory for the diagnosis of amoebiasis. The IC assay revealed a detection limit of 10³ trophozoites/g faeces and no cross-reactivity with other parasites and failed to detect *E. histolytica* antigen in frozen faeces. In our laboratory located in a non-endemic setting this assay could not replace the methods currently used for the diagnosis of amoebiasis.

*Entamoeba histolytica*, the aetiological agent of amoebiasis, is the major cause of parasitic dysentery with faecal-oral route transmission in developing countries (second only to malaria as leading cause of protozoan diseases worldwide), with approximately 50 million cases and 100,000 deaths annually (Tanyuksel and Petri, 2003). In industrialised countries, travellers from regions that are endemic for the infection and men who have sex with men are at higher risk than the general population of acquiring *E. histolytica* infections (Verkerke et al., 2015). Detection of *E. histolytica* is an important goal of the clinical parasitology laboratory because the identification of *E. dispar* as a morphologically identical but non-pathogenic species has highlighted the need for non-microscopic detection methods able to differentiate between the two organisms (Calderaro et al., 2015). DNA detection by PCR is the method of choice for the discrimination between *E. histolytica* and *E. dispar* ([www.cdc.gov](http://www.cdc.gov)), and it is the most sensitive and specific method, overcoming the limitations of microscopy and culture (Blessmann et al., 2002; Korpé et al., 2012; Calderaro et al., 2015). Antibody detection lacks sensitivity in early disease and it is unable to distinguish active infection from previous exposure (Korpé et al., 2012). Antigen detection in stools has been proved more sensitive than microscopy but is less sensitive than DNA-based methods (ElBakri et al., 2013). Moreover, many tests cannot reliably distinguish between *E. histolytica* and *E. dispar* (Korpé et al., 2012).

In our laboratory, located in a non-endemic area for amoebiasis (4 cases out of a total of 8,886 patients with suspected intestinal parasitosis during 2006-2010) (Calderaro et al., 2014), the diagnosis of amoebiasis is performed by microscopic examination, culture for intestinal protozoa and real-time PCR for the differentiation of *E. histolytica* and *E. dispar* (Calderaro et al., 2014).

In this prospective study, a field evaluation of the immunochromatographic (IC) assay “TECHLAB® *E. HISTOLYTICA QUIK CHEK™*” (Techlab, Blacksburg, VA, USA) for the detection of *E. histolytica* in fresh or frozen stool samples was performed and compared with that of the methods currently used in our laboratory for the diagnosis of amoebiasis.

This study included 6 xenic cultures (*E. histolytica* 8026, 373, 2940, 1656, 1337 and *E. dispar* 2550 strains) (Calderaro et al., 2015), 1 axenic culture (*E. moshkovskii* Laredo reference strains) (Calderaro et al., 2015) and 36 clinical samples. Among the 36 faecal samples 19 were selected among those sent to our laboratory for the diagnosis of intestinal parasitosis during April-May 2015 and on February 2016 and 17 belonged to our collection stored at -20°C: 4 positive for *E. histolytica + Blastocystis hominis* (2), *E. histolytica + B. hominis + Ascaris lumbricoides + Ankylostoma duodenale* (1), and *E. dispar* (1), respectively, and 13 negative for intestinal parasites. All the faecal samples were subjected to microscopic examination (wet mounts) for intestinal parasites including formalin-ethyl acetate sedimentation technique according to Ritchie (Ritchie, 1948) and cultivation in Robinson’s medium for protozoa (Calderaro et al., 2006; Calderaro et al., 2014). The DNA extraction and the real-time PCR assays for the identification of *E. histolytica* and *E. dispar* from cultures and from all the clinical samples were performed as previously described (Calderaro et al., 2006).
For the IC assay, an aliquot of faecal samples (2 mm in diameter of solid samples or 25 μl for liquid samples) or 25 μl of cultures (10⁴ trophozoites/ml) were respectively added to 500 μl of diluent in test tubes according to the manufacturer’s instructions. Positive and negative controls were included in the kit. The detection limit of the IC assay was calculated by using 2 faecal samples negative for *E. histolytica* and *E. dispar* (by real-time PCR assay) experimentally seeded with trophozoites of *E. histolytica* strain 8026 and strain 1337, respectively. Serial ten-fold dilutions (from 10⁶ -10⁴ trophozoites/ml) were vortexed with an equal volume of sterile culture medium containing 1 g of faeces and 25 μl of these were submitted to the assay as described above. As expected, the analytical sensitivity of the IC assay was 100 times lower than that of the real-time PCR assay (10³ vs 10 trophozoites/g of faeces, respectively). The IC test was also performed on the same dilutions described above to test the detection limit and frozen at -20°C and the analytical sensitivity observed was 10⁵ trophozoites/g of faeces. Moreover, the IC assay was evaluated on additional 2 experimentally seeded samples, containing cysts and trophozoites of the *E. histolytica* strains (N. 373, 2940), tested both fresh and frozen (72 h at -20°C), showing a positive result only when the fresh samples were analysed. A negative result by the IC assay was obtained when the negative control was used (*Figure 1A*), and a positive result was obtained when the positive control was used (*Figure 1B*). Interestingly, although the kit was not validated for culture, the specific positive band was observed by using the *E. histolytica* strains 8026, 373, 2940 and, 1337 cultivated in xenic Robinson’s medium (*Figure 1D*) and a negative result was obtained analysing the *E. dispar* 2550 (*Figure 1C*) and the *E. moshkovskii* Laredo cultures, respectively. Only one xenic culture (*E. histolytica* strain 1656) resulted negative by IC. Although this strain had been identified as *E. histolytica* by PCR and sequencing (Calderaro et al., 2015), it has an anomalous behaviour as demonstrated in a previous study in which it did not show specific *E. histolytica* markers (Calderaro et al., 2015). Among the 19 fresh faecal samples submitted to the methods routinely used for the diagnosis of intestinal parasitosis (Calderaro et al., 2014), 5 were negative and 14 were positive for *E. dispar* (3), *B. hominis* (3), *Entamoeba. coli* (1), *Giardia intestinalis* (1), *Strongyloides stercoralis* (1), *Taenia saginata* (1), *E. coli + B. hominis* (1), *Dientamoeba fragilis + B. hominis* (2), respectively and 1 sample
was positive for *B. hominis* and the intestinal spirochae *Brachyspira pilosicoli*. The results of the IC assay performed on the 36 faecal samples included in this study are reported in Table 1. No cross-reactivity was observed when intestinal parasites other than *E. histolytica*, including *E. dispar, E. coli, B. hominis, G. intestinalis, D. fragilis, S. stercoralis*, and *T. saginata*, and the intestinal spirochaete *pilosicoli* were found in 13 faecal samples. With the remaining faecal sample positive for *B. hominis* the IC assay resulted invalid (twice) and the PCR assay excluded both the presence of *E. histolytica* and *E. dispar*. In this case, the use of the IC assay alone would not have led to a definitive result. Intra-sample accuracy was evaluated performing the IC assay in 2 separate sections of our laboratory by testing 2 experimentally seeded faecal samples with *E. histolytica* strains 8026 and 1337 in 3 different days by 2 different operators, confirming the 100% correlation already reported by the manufacturer.

In our hands, unlike the results obtained by other research groups (Korpe et al., 2012; Verkerke et al., 2015) freezing for a long time could affect the results. In fact, negative results were obtained when the IC assay was applied on the two frozen samples positive for *E. histolytica* by real-time PCR. Only in one case the specific band of *E. histolytica* appeared after 30 min, over the time indicated by the manufacturer. These results are not unexpected on the basis of the manufacturer’s indications which reported that frozen specimens may lose reactivity due to the freezing and thawing. In any case, this limit should be overcome by the fact that all the samples routinely analysed in our laboratory for the diagnosis of amoebiasis are fresh. The availability of only two positive for *E. histolytica* stored samples is representative of the epidemiology of our area (Calderaro et al., 2006; Calderaro et al., 2014).

Although in our experience the IC assay proved to be rapid, less cumbersome and less expensive than the real-time PCR assay, its usefulness for the routine diagnosis of amoebiasis was not demonstrated, taking into account the scarcity of samples positive for *E. histolytica* in our area. Furthermore, the poor sensitivity observed in our hands does not ensure *E. histolytica* can be detected when the parasite is present at 10^7 trophozoites/g, making use of the IC assay not advisable in non-endemic settings. In our laboratory the low occurrence of the infection by *E. histolytica* has been successfully detected to date by the combination of microscopy, culture and real-time PCR. Furthermore, in contrast to PCR, the IC assay aimed to reveal *E. histolytica* but it is not able to detect the morphologically identical *E. dispar* (Korpe et al., 2012).

The identification of *E. histolytica* and its differentiation from *E. dispar* is essential in order to administer prompt and targeted therapy to patients only infected with the pathogenic amoeba (Calderaro et al., 2006) and to provide for proper food hygiene even in cases of infection with *E. dispar*. This aspect is also essential as a recent study revived the possibility that *E. dispar* can produce lesions in humans (Oliveira et al., 2015).

Thus, taking into account this consideration and the results of this study, if available and accessible the real-time PCR assay is the most accurate tool for the diagnosis of amoebiasis. Van de Bosche and colleagues (2015) reported the usefulness of rapid detection tests (RDT), such as IC, in areas where the prevalence of amoebiasis is high and resources are poor. Conversely, for low endemic settings they suggested the use of RDT as a rapid screening method prior to microscopic examination, to be performed only when the exclusion of other pathogens becomes necessary. In this algorithm, the differentiation between *E. histolytica* and *E. dispar* by PCR remains necessary (Van den Bosche et al., 2015).

In this light, the application of the IC assay should be evaluated individually by every single laboratory, depending on the cost/effectiveness related to the prevalence of the infection and, as already reported, to the population investigated and the availability of personnel skills (Van den Bosche et al., 2015).

On the basis of our experience in a laboratory located in a non-endemic setting where the application of a PCR assay should be included in the diagnostic algorithm of amoebiasis, the IC assay was not suitable for this aim.

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**Author contribution**

Conceived and designed the experiments: AC

Performed the experiments: MP, SR, MB

Analyzed the data: AC, MCA, MCM, CC, FDC

Contributed reagents/materials/analysis tools: AC

Wrote the paper: AC, MP, GP, SM, MB

**References**


www.cdc.gov.