ENTAMEBA Ag CARD

For in *Vitro* diagnostic use only

In vitro diagnostic rapid test for the qualitative detection of *Entamoeba spp.* antigens in human faeces including positive and negative controls

I. INTRODUCTION AND INTENDED USE

Amebiasis is the infection of the human gastrointestinal tract by *Entamoeba histolytica*, a protozoan parasite that is capable of invading the intestinal mucosa and may spread to other organs, mainly the liver. *Entamoeba dispar*, an ameba morphologically similar to *E. histolytica* that also colonizes the human gut, has been recognized recently as a separate species with no invasive potential. The acceptance of *E. dispar* as a distinct but closely related protozoan species has had profound implications for the epidemiology of amebiasis, since most asymptomatic infections found worldwide are now attributed to this noninvasive ameba.
Invasive amebiasis due to *E. histolytica* is more common in developing countries.
The **Entameba Ag card** is a rapid chromatographic immunoassay for the qualitative detection of *Entamoeba spp.* antigens in human faeces specimens to aid in the diagnosis of amoebiasis.

II. PRINCIPLE OF THE TEST

The **Entameba Ag card** Mascia Brunelli is a qualitative lateral flow immunoassay for the detection of *Entamoeba spp.* antigen in human feces samples. The membrane is pre-coated with monoclonal and polyclonal antibodies against *Entamoeba* antigens on the test line region. During testing, the sample reacts with the particle coated with anti- *Entamoeba* antibodies which was pre-dried on the test strip. The mixture moves upward on the membrane by capillary action. In the case of a positive result the specific antibodies present on the membrane will react with the mixture conjugate and generate a coloured line. A green coloured band always appears in the control line and serves as verification that sufficient volume was added, that proper flow was obtained and as an internal control for the reagents.

III. REAGENTS AND MATERIALS

Each kit contains material for 10 determinations:
Entameba Ag card (N.10) cassettes packaged with a desiccant in individual aluminum pouches
Diluent –Extraction buffer (10x1.0 mL tubes LIQ.EXTR) Sample Diluent of buffer containing proteins and preservative
Positive Control: N.1 vial with dropper containing non-infectious components, sodium azide (NaN_3) as preservative (1x0.5 mL).
Negative Control: use Extraction buffer-Diluent
Instruction for use

Required materials (not supplied)

Specimen collection container, Disposable gloves and container, Plastic pipettes and Timer.

IV. SPECIAL PRECAUTIONS

- The kit is for professional use and for in vitro diagnosis only.
- Do not use after expiration date. Do not use the test if pouch is damaged.
- The test should remain in the sealed pouch until use.
- All operations linked to the use of the test must be performed in accordance with Good Laboratory Practices.
- All the specimens should be considered potentially hazardous and handled in the same manner as an infectious agent.
- The test should be discarded in a proper biohazard container after testing.
- The test must be carried out within 2 hours of opening the sealed bag.

V. STORAGE AND STABILITY

Store as packaged in the sealed pouch either at refrigerated or room temperature (2-30ºC/36-86ºF). The test is stable through the expiration date printed on the sealed pouch. The test must remain in the sealed pouch until use. Do not freeze.

VI. SPECIMEN COLLECTION AND PREPARATION

Collect sufficient quantity of feces (1-2 g or mL for liquid sample). Stool samples should be collected in clean and dry containers (no preservatives or transport media). The samples can be stored in the refrigerator (2-8ºC/36-46.4ºF) for 24-48 hours prior to testing. For longer storage the specimen must be kept frozen at –20ºC/-4ºF. In this case, the sample needs to be totally thawed, and brought to room temperature before testing.

VII. ASSAY PROCEDURE

Review “specimen collection” instructions. Do not open pouches until ready to perform the assay. Make sure that all reagents are at room temperature before beginning the assay.

Process the collected stool samples

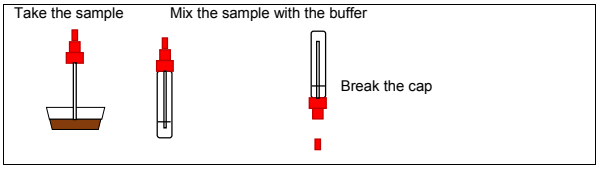
Use a separate swab or stick, dropper and testing tube or vial for each sample. Unscrew the top of the extraction buffer tube. Collect the stool sample with the tip of the collection device by dipping in three different places of the same stool specimen. Verify to transfer a small portion (150-250 mg) of stool. Put the collection device back into the plastic test tube. Shake the extraction tube in order to get an homogeneous solution. For liquid or semi-solid stools, withdraw the sample using a separate pipette. Dispense 250 µl of each stool into an extraction tube. Mix carefully, than vortex 15 seconds.

Test Procedure

Allow the tests, stool samples and buffer to reach to room temperature (15-30ºC/59-86ºF) prior to testing. Do not open pouches until ready to perform the assay.

1. Remove the Entameba card from its sealed pouch and use it as soon as possible. Use a separate device for each sample.
2. Break the cap of the tube
3. Dispense 3-4 drops or 100 uL into the specimen well. Start the timer.
4. Read the result at **10 minutes** after dispensing the sample.

Procedure for the controls: For the Positive and Negative Control use the same procedure (from step 3 onwards)

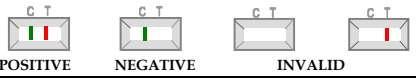


VIII. INTERPRETING THE RESULTS

POSITIVE: In addition to the GREEN control band across the central window in the site marked with the letter C (control line), a RED band (test line) also appears in the site marked with the letter T (result region). The sample is positive for Entamoeba.

NEGATIVE: Only one GREEN control band appears across the central window in the site marked with the letter C (control line).

INVALID: A total absence of the control coloured band. Insufficient specimen volume, incorrect procedural techniques or deterioration of the reagents are likely the reasons for control line failure. Review the procedure and repeat the tests using a new test.



The intensity of the blue coloured band in the result line region will vary depending on the concentration of antigens in the specimen. However, neither the quantitative value, nor the rate of increase in antigens can be determined by this qualitative test.
Internal procedural controls are included in the test: A green line appearing in the control line region (C). It confirms sufficient specimen volume and correct procedural technique.

IX. PERFORMANCE

A) Expected values

Entamoeba histolytica infects more than 500 million people worldwide. Almost from the time of its discovery, it was observed that although E. histolytica most often causes mild or asymptomatic infections, around 10% of patients develop severe dysentery and life-threatening invasive and extraintestinal disease. 70,000 people are estimated to die each year from amebic colitis and amebic liver abscess.Dysenteric and diarrheic syndromes account for 90% of cases of invasive intestinal amebiasis.

B) Sensitivity and Specificity

Stool samples from patients of different Hospitals were studied. The result using **Entameba Ag card** and compared with other immunochromatographic test showed: Sensitivity: >99% of sensitivity and >99%of sensitivity

C) Cross-Reactivity

An evaluation to determine the cross reactivity of **Entameba Ag card** was performed. There is no cross reactivity with common intestinal pathogens, other organisms and substances occasionally present in faeces: *Escherichia coli, Campylobacter, Clostridium difficile, Giardia lamblia, H. pylori, Listeria monocytogenes, Salmonella, Shigella.*

X. LIMITS OF THE KIT

1. ***Entameba* Ag card** will only indicate the presence of parasites in the specimen (qualitative detection) and should be used for the detection of *Entameba* antigens in faeces specimens only. Neither the quantitative value nor the rate of increase in antigen concentration can be determined by this test.
2. An excess of sample could cause wrong results (brown bands appear). Dilute the sample with the buffer and repeat the test.
3. If the test result is negative and clinical symptoms persist, additional testing using other clinical methods is recommended. A negative result does not at any time preclude the possibility of *Amebiasi* infection.
4. After one week of infection, the number of parasites in faeces is decreasing, making the sample less reactive. Stool samples should be collected within one week of the onset of symptoms.
5. This test provides a presumptive diagnosis of infection caused by *Entameba*. All results must be interpreted together with other clinical information and laboratory findings available to the physician.
6. In the case of positive result, further tests are needed to confirm the presence of *E.Histolica* and suggest the appropriate therapy.

XI. REFERENCES

1- REED S., et al. “Cloning of a Virulence Factor of Entamoeba histolytica”. *Journal of Clinical Investigation*, Volume 91, April 1993, 1532-1540.
2- HAQUE R., et al. “Diagnosis of Amebic Liver Abscess and Intestinal Infection with the TechLab *Entamoeba histolytica* II Antigen Detection and Antibody Tests”. *Journal of clinical microbiology*, Sept. 2000, p. 3235–3239.
3- ESPINOSA-CANTELLANO M., et al. “Pathogenesis of Intestinal Amebiasis: From Molecules to Disease”. *Clinical Microbiology Reviews*, Apr. 2000, p. 318–331.

IVD	In Vitro Diagnostic Medical Device		Temperature limitation	LOT	Batch code (EXXX)		Manufacturer		Keep dry	NON STERILE	Non-sterile
	Consult Instructions for use		Use by (year/month)	REF	Catalogue number		Do not reuse		Fragile, handle with care		Keep away from heat

CONTENT	**COD. VC1030 (10 tests)**
Entameba Ag Card	10 items
Liq.Extr tubes	10 x 1.0 mL
Positive Control	1 x 0.5 mL
Instruction for use	1 item

EDMA (EDMS) CODE 1505100100 CE IVD