



REF 6004

May 31, 2007

# Entamoeba histolytica Antigen

- 96 determinations -



IVD *In vitro* diagnostic device

Enzyme immunoassay for the determination of *Entamoeba histolytica* (pathogenic strain) in fecal specimens

<b>REF</b>	Catalogue number	<b>LOT</b>	Batch code
	Consult accompanying documents		Manufactured by
	Temperature limitation		Use by
	Consult operating instruction		Biological risk



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## INTENDED USE

*Entamoeba histolytica* Antigen is used for the qualitative determination of *Entamoeba histolytica* Antigen in fecal specimens.

*Entamoeba histolytica* is the causative agent of amoebiasis (amoebic dysentery, amoebic liver abscess). Only trophozoites of virulent strains invade the intestinal wall and cause ulcers, which produce raspberry jelly like blood and mucous in stools. Amoeba which reach the liver via the enteral vascular system cause extensive abscesses (1). The infectious cysts of this protozoon reach the intestine via the fecal-oral route through food or water contaminated with feces. The vegetative trophozoites are released by excystation and multiply by bisection. Meanwhile molecular biological (PCR) and biochemical (iso-enzyme analysis) methods confirm the classification of the pathogenic, invasive strains as separate species *Entamoeba histolytica*. The non-invasive strains are classified as *Entamoeba dispar*. The two species share an identical morphology and therefore a differentiation by microscopy is not possible. The microscopic investigation of stool specimens additionally requires experience, is time consuming and not suited as screening method (1-6).

*Entamoeba histolytica* releases specific antigens into the intestine during its life cycle. These antigens are excreted with the feces of the infected persons. The antigen detection by enzyme immunoassay can serve as specific and easy to perform alternative to microscopy.

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2. Gonzalez-Ruiz, a. et al. (1994): „Diagnosis of Amebic Dysentery by Detection of *Entamoeba histolytica* Fecal Antigen by an Invasive Strain-Specific, Monoclonal Antibody-Based Enzyme-Linked Immunosorbent Assay“. Journal Of Clinical Microbiology, Vol.32, No. 4, p. 964-970
3. Blakely, P., Sargeant, P.G. and Reed, S.L. (1990): „An Immunogenic 30-kDa Surface Antigen of Pathogenic Clinical Isolates of *Entamoeba histolytica*“. The Journal of Infectious Diseases 162: 949-954
4. Stanley S. L. et al. (1995): „The Serine-rich *Entamoeba histolytica* Protein Is a Phosphorylated Membrane Protein Containing O-Linked Terminal N-Acetylglucosamine Residues“. The Journal of Biological Chemistry Vol. 270, No. 8, p. 4121-4126
5. Tachibana H. et al. (1991): „Differences in genomic DNA Sequences between Pathogenic and Nonpathogenic Isolates of *Entamoeba histolytica* Identified by Polymerase Chain Reaction“. Journal Of Clinical Microbiology Vol. 29, No. 10, p. 2234-2239
6. Mirelman, D., Nuchamowitz, Y. and 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*“. Journal Of Clinical Microbiology Vol. 35, No. 9, p. 2405-2407

## PRINCIPLE OF THE TEST

*Entamoeba histolytica* Antigen is a fast enzymometric two-step immunoassay for the qualitative determination of *Entamoeba histolytica* employing polyclonal solid phase immobilized and horseradish peroxidase (HRP) labeled antibodies (rabbit) to two different epitopes of the serine-rich 30 kDa membrane protein (SREHP) only found in pathogenic *Entamoeba histolytica* strains.

*Entamoeba histolytica* antigens of specimens and the positive control react with anti-*Entamoeba histolytica* peptide 1 antibodies coated on the solid phase of the microplate during the first incubation step. After incubation for 30 minutes at room temperature (RT) non-bound material is removed by a wash step.

Bound *Entamoeba histolytica* antigens react specifically with anti-*Entamoeba histolytica* peptide 2 F(ab)<sub>2</sub> conjugated to HRP. After an incubation period of 30 min at RT non-bound components are separated from the solid-phase immune complexes formed by the following wash step.

HRP converts the colorless substrate solution of 3,3',5,5'-tetramethylbenzidine (TMB) added into a blue product. The enzyme reaction is stopped by dispensing an acidic solution into the wells after 10 min at room temperature turning the solution from blue to yellow.

The optical density (OD) of the solution read at 450 nm is directly proportional to the amount of *Entamoeba histolytica* antigen bound. For optimal results a reference filter (620 nm wavelength) should be used. Considering the cut off value results are interpreted as positive or negative.

## Specimen collection and storage

The *Entamoeba histolytica* Antigen ELISA is intended for the detection of *Entamoeba histolytica* in 1:11 externally diluted stool specimens (100 mg stool in 1.0 ml sample diluent (C)). Rectal swabs should be suspended in 1 ml sample diluent by pressing the swab to the inner wall of the tube several times (make sure that the sample volume is sufficient). Mix samples thoroughly, e. g. on a vortex. If necessary sediment floating particles of the homogenous suspension by centrifugation in a micro-centrifuge (e. g. Eppendorf) for 1 minute at maximum speed. Fecal samples should be collected into containers that do not contain preservatives, metal ions or oxidizing agents.

## Preparation before use

Allow frozen or refrigerated fecal samples to reach room temperature prior to assay. Take care to agitate samples gently in order to ensure homogeneity.

The storage time at 2-8°C should not exceed 48 hours. Long-term storage requires - 20 °C. Repeated freezing and thawing of samples should be avoided.

### TEST COMPONENTS FOR 96 WELLS

<b>A</b> <b>Ag</b> <b>96</b>	<b>Microtiter plate</b> , 12 breakable strips per 8 wells coated with polyclonal antibodies to <i>Entamoeba histolytica</i> peptide 1 (rabbit)	1 vacuum sealed with desiccant
<b>B</b> <b>BUF</b> <b>WASH</b> <b>10x</b>	<b>Concentrated wash buffer</b> sufficient for 1000 ml solution	100 ml concentrate capped white
<b>C</b> <b>DIL</b>	<b>Sample diluent</b>	100 ml ready for use capped black
<b>D</b> <b>CONJ</b>	<b>Conjugate</b> Containing polyclonal anti-SREHP-Peptid Antibodies (IgG/F(ab) <sub>2</sub> sheep) coupled with HRP	12 ml ready for use capped brown
<b>E</b> <b>SOLN</b> <b>TMB</b>	<b>Substrate</b> 3,3',5,5'-tetramethylbenzidine in citrate buffer containing hydrogen peroxide	15 ml ready for use capped blue
<b>F</b> <b>H2SO4</b> <b>0.25 M</b>	<b>Stop solution</b> 0.25 sulfuric acid	15 ml ready for use capped yellow
<b>P</b> <b>CONTROL</b>	<b>Positive control</b> <i>Entamoeba histolytica</i> positive specimen (inactivated) <b>+</b>	2.0 ml ready for use capped red
<b>N</b> <b>CONTROL</b>	<b>Negative</b> <i>Entamoeba histolytica</i> negative specimen <b>-</b>	2.0 ml ready for use capped green

## Materials required but not provided

- micropipettes
- multi-channel pipette or multi-pipette trough for multi-channel pipette
- 8-channel wash comb with vacuum pump and waste bottle or microplate washer
- distilled or de-ionized water
- glassware

## Size and storage

*Entamoeba histolytica* Antigen has been designed for 96 determinations.

The expiry date of each component is reported on its respective label that of the complete kit on the box labels.

Upon receipt, all components of the *Entamoeba histolytica* Antigen have to be kept at 2 - 8 °C, preferably in the original kit box.

After opening all kit components are stable for at least 2 months, provided proper storage.

## Preparation before use

Allow all components to reach room temperature prior to use in the assay.

The microtiter plate is vacuum-sealed in a foil with desiccant. The plate consists of a frame and strips with breakable wells. Allow the sealed microplate to reach room temperature before opening. Unused wells should be stored refrigerated and protected from moisture in the original cover carefully resealed.

Prepare a sufficient amount of wash solution by diluting the concentrated wash buffer 10 times with de-ionized or distilled water. For example, dilute 5 ml of the concentrate with 45 ml of distilled water per strip. The wash solution prepared is stable at 2 - 8 °C up to 30 days.

Make sure the soak time of the wash buffer in the wells is at least 5 seconds per wash cycle.

Avoid exposure of the TMB substrate solution to light!

### ASSAY PROCEDURE

- Dilute samples with sample diluent (C) 1 + 10 (w/v), e.g. 100 mg stool + 1 ml sample diluent (C)
- Avoid any time shift during pipetting of reagents and samples.

1. Bring all reagents to room temperature (20-25°C) before use. Mix gently without causing foam.
2. Dispense **2 drops** negative control (N) **2 drops** positive control (P) **100 µl** diluted samples
3. Seal plate, incubate **30 min** at room temperature (20-25°C).
4. Decant, then wash each well **five** times using **300 µl** wash solution (made of B).
5. Dispense **two drops** conjugate (D) into the respective wells
6. Seal plate, incubate **30 min** at room temperature (20-25°C).
7. Decant, then wash each well **five** times using **300 µl** wash solution (made of B).
8. Add **2 drops** of substrate (E) to each well.
9. Incubate **10 min protected from light** at room temperature (20-25°C).
10. Add **2 drops** of stop solution (F) to each well and mix gently.
11. Read the OD at **450 nm** versus 620 or 690 nm within **30 min** after adding the stop solution.

## DATA PROCESSING

### Qualitative evaluation

### Cut-off determination

OD of the negative control + 0.2 OD units

## REFERENCE VALUES

Entamoeba histolytica Antigen	
Negative	≤ cut-off
Positive	> cut-off

### Example of typical assay results

wells	OD (a)	OD (b)	OD (mean)
Negative control	0.101	0.111	0.106
Positive control	2.826	2.844	2.835
Positive	> 0.106 + 0.200		= 0.306
Specimen 1	2.318	2.286	2.302 - positive
Specimen 2	0.116	0.126	0.121 - negative

### Test validity

The test run is valid if:

- the mean OD of the negative control is ≤ 0.20
- the mean OD of the positive control is ≥ 1.00

If the above mentioned quality criteria are not met, repeat the test and make sure that the test procedure is followed correctly (incubation times and temperatures, sample and wash buffer dilution, wash steps etc.). In case of repeated failure of the quality criteria contact your supplier.

### Limitations of the method

Cross contamination of reagents and samples can produce false results. Incorrect dilutions, not sufficiently homogenized samples or solid particles after centrifugation of the suspension can cause false-negative as well as false-positive results

Fermented samples with pH values below 5 after re-suspension may produce false negative results.

Due to the susceptibility of the antigen to proteases samples should be tested within 24 hours when stored at 2 - 8 °C. In case of longer storage times freezing at - 20 °C is recommended. Repeated freezing and thawing of samples should be avoided. Stool specimens with preservatives like formalin cannot be tested in the Entamoeba histolytica Antigen ELISA. It is recommended to take samples for dilution from two different areas of a stool specimen, because of the possibly non-homogenous antigen distribution.

The final result interpretation should consider clinical findings as well.

## CHARACTERISTIC ASSAY DATA

### Precision

Intra-assay coefficient of variation (c. v.) in the Entamoeba histolytica Antigen ELISA calculated from 12fold determinations of the samples:

sample	OD mean	SD	c. v. (%)
I.	0.463	0.024	5.3
II.	0.620	0.040	6.4
III.	1.208	0.078	6.5
IV.	1.841	0.137	7.4

Inter-assay coefficient of variation (c. v.) in the Entamoeba histolytica Antigen ELISA in 10 different test runs calculated from 3fold determinations of the samples:

sample	OD mean	SD	c. v. (%)
I.	0.409	0.019	4.68
II.	0.968	0.074	7.7
III.	1.647	0.122	7.4
IV.	2.720	0.128	4.7

### Lower detection limit

The lower detection limit of Entamoeba histolytica antigens in the Entamoeba histolytica Antigen ELISA was determined by titration of fecal samples spiked with Entamoeba obtained from culture.

The lower detection limit of Entamoeba histolytica was determined at  $5 \times 10^3$  to  $6 \times 10^3$  trophozoites per ml of diluted fecal sample.

Fecal samples spiked with *Entamoeba dispar* did not show any positive reaction in the Entamoeba histolytica Antigen ELISA up to concentrations of  $> 10^5$  trophozoites per ml of diluted fecal sample.

### Cross reactivity

Fecal samples positive for one of the following intestinal parasites and non pathogenic amoeba resp., did not show any cross reaction in the Entamoeba histolytica Antigen ELISA:

**Giardia lamblia**  
**Cryptosporidium sp.**  
**Entamoeba dispar**  
**Entamoeba coli**  
**Dientamoeba fragilis**  
**Entamoeba hartmanni**

### REMARKS:

## INCUBATION SCHEME

# Entamoeba histolytica Antigen (6004)

**Dilute patients sample                      100 mg sample + 1 ml sample diluent (C)**

1	<b>Bring all reagents to room temperature (20-25°C)</b>		
2	Dispense	Negative control (N) Positive control (P) 1 + 10 (w/v) prediluted samples	2 drops 2 drops 100 µl
3	Seal plate and incubate <span style="float: right;">30 min, room temperature (20-25°C)</span>		
4	Wash <span style="float: right;">Decant, 5 x 300 µl wash solution (made of B)</span>		
5	Dispense conjugate (D)		2 drops
6	Seal plate and incubate <span style="float: right;">30 min, room temperature (20-25°C)</span>		
7	Wash <span style="float: right;">Decant, 5 x 300 µl wash solution (made of B)</span>		
8	Dispense substrate (E)		2 drops
9	Incubate protected from light <span style="float: right;">10 min, room temperature (20-25°C)</span>		
10	Dispense stop solution (F)		2 drops
11	Read at 450 nm against 620 (690) nm within 30 min.		

### SAFETY PRECAUTIONS

- **This kit is for in vitro use only.** Follow the working instructions carefully. GA GENERIC ASSAYS GmbH and its authorized distributors shall not be liable for damages indirectly or consequentially brought about by changing or modifying the procedure indicated. The kit should be performed by trained technical staff only.
- The expiration dates stated on the respective labels are to be observed. The same relates to the stability stated for reconstituted reagents.
- Do not use or mix reagents from different lots.
- Do not use reagents from other manufacturers.
- Avoid time shift during pipetting of reagents.
- All reagents should be kept at 2 - 8 °C before use in the original shipping container.
- Some of the reagents contain small amounts of Thimerosal (< 0.1 % w/v) and Kathon (1.0 % v/v) as preservative. They must not be swallowed or allowed to come into contact with skin or mucosa.
- Source materials derived from human body fluids or organs used in the preparation of this kit were tested and found negative for HBsAg and HIV as well as for HCV antibodies. However, no known test guarantees the absence of such viral agents. Therefore, handle all components and all patient samples as if potentially hazardous.
- Since the kit contains potentially hazardous materials, the following precautions should be observed:
  - Do not smoke, eat or drink while handling kit material,
  - Always use protective gloves,
  - Never pipette material by mouth,
  - Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.