



# INSTRUCTION MANUAL

REF 6005

February 25<sup>th</sup>, 2004

## Giardia lamblia Antigen

- 96 determinations -



IVD *In vitro* diagnostic device

Enzyme immunoassay for the determination of *Giardia lamblia* (*Lamblia intestinalis*) 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

*Giardia lamblia* Antigen is used for the qualitative determination of *Giardia lamblia* (*Lamblia intestinalis*) Antigen in fecal specimens.

*Giardia lamblia* (*Lamblia intestinalis*) is one of the most common human intestinal protozoan pathogens world-wide. The incidence strongly depends on the geographic region and reaches 2-7 % in central Europe and exceeds 50 % in tropical countries (1, 2).

The life cycle of *Giardia lamblia* is characterized by two stages: the trophozoite and the cyst stage. The trophozoite is the motile dividing stage and inhabits the upper small intestine. Ascending infections of the gallbladder may also occur. The cyst is the infective form of the parasite. It develops in the intestine and is excreted with the feces. Cysts are transmitted via contaminated food or drinking water but also from person to person (3). The clinical picture of a *Giardia lamblia* infection ranges from the asymptomatic carrier state to acute diarrhea, which is often accompanied by abdominal pain and flatulence. Chronic giardiasis can cause severe mal-absorption syndrome (1, 2).

Giardiasis is usually diagnosed by microscopic detection of trophozoites and/or cysts in fecal smears after commonly used staining techniques or direct immune fluorescence. These methods are time-consuming, require trained personnel and can only detect parasites with intact morphology. Immunologic methods like enzyme immunoassays detecting *Giardia lamblia* antigens may overcome these problems (4).

1. Murray, P.R. (Chief Editor): Manual of Clinical Microbiology. ASM Press, Washington D.C. Sixth Edition 1995
2. Wolfe, M. S. (1990): Giardiasis (Review). Clinical Microbiology Reviews 5 (1), 93-100
3. Faubert, G. (2000): Immune Response to *Giardia duodenalis*. Clinical Microbiology Reviews 13 (1), 35-54
4. Janoff, E. N. et al. (1992): Diagnosis of *Giardia lamblia* Infections by Detection of Parasite Specific Antigens. Journal of Clinical Microbiology 27, 431-435

### PRINCIPLE OF THE TEST

*Giardia lamblia* Antigen is a fast enzymometric two-step immunoassay for the qualitative determination of *Giardia lamblia* antigen employing polyclonal solid phase immobilized and horseradish peroxidase (HRP) labeled antibodies (rabbit) to *Giardia lamblia*.

*Giardia lamblia* antigens of specimens and the positive control react with anti-*Giardia lamblia* 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 *Giardia lamblia* antigens react specifically with anti-*Giardia lamblia* antibodies 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 *Giardia lamblia* 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 *Giardia lamblia* Antigen ELISA is intended for the detection of *Giardia lamblia* 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>Giardia lamblia</i> antigen (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 anti- <i>Giardia lamblia</i> IgG- (rabbit) 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>Giardia lamblia</i> antigen positive specimen (inactivated) <b>+</b>	2.0 ml ready for use capped red
<b>N</b> <b>CONTROL</b>	<b>Negative</b> <i>Giardia lamblia</i> antigen 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

*Giardia lamblia* 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 *Giardia lamblia* 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

Giardia lamblia Antigen	
Negative	≤ cut-off
Positive	> cut-off

### Example of typical assay results

wells	OD (a)	OD (b)	OD (mean)
Negative control	0.108	0.100	0.104
Positive control	2.816	2.834	2.825
Positive	> 0.104 + 0.200		= 0.304
Specimen 1	2.218	2.186	2.202 - positive
Specimen 2	0.118	0.126	0.122 - 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 ≥ 0.80

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

There is no correlation between the measured OD and the severity of the disease and the OD of samples should not be correlated to the OD of the test controls.

Cross-contamination of reagents and samples can produce false results. Incorrect dilutions, not sufficiently homogenized samples and samples, which stayed for sedimentation for more than 10 minutes can cause false results. Diluted samples, which stayed for more than 10 minutes should be mixed again before testing.

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

A negative ELISA result does not exclude a *Giardia lamblia* infection, because the excretion of cysts is periodic. Thus at least one further stool specimen of the regarding person should be demanded in case of a negative test result but clinical suspect.

Clinical findings have to be considered for a final interpretation of the test results.

## CHARACTERISTIC ASSAY DATA

### Precision

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

sample	OD mean	SD	c. v. (%)
I.	1.438	0.099	6.9
II.	1.007	0.065	6.4
III.	0.673	0.043	6.4
IV.	0.243	0.012	5.0

Inter-assay coefficient of variation (c. v.) in the *Giardia lamblia* Antigen ELISA in 11 different test runs calculated from 3fold determinations of the samples:

sample	OD mean	SD	c. v. (%)
I.	1.488	0.071	4.8
II.	1.034	0.048	4.6
III.	0.656	0.051	7.8
IV.	0.246	0.027	10.9

### Lower detection limit

The lower detection limit of *Giardia lamblia* antigens in the *Giardia lamblia* Antigen ELISA was determined by titration of fecal samples spiked with *Giardia lamblia* cysts and cultured trophozoites.

The lower detection limit of *Giardia lamblia* Antigen was determined 5 x 10<sup>3</sup> cysts and 2 x 10<sup>4</sup> trophozoites per ml of diluted fecal sample.

### Clinical evaluation

A total of 409 stool specimens were tested in parallel with the *Giardia lamblia* Antigen ELISA and immunofluorescence (IF).

	IF	
	Positive	Negative
Giardia lamblia Antigen ELISA	39	2
	1	367

Specificity: 99.5 %

Sensitivity: 97.5 %

### Cross reactivity

Fecal samples positive for one of the following intestinal parasites and other respective pathogens did not show any cross reaction in the *Giardia lamblia* Antigen ELISA:

**Adenovirus, Ancylostoma duodenale, Ascaris lumbricoides, Blastocystis hominis, Cryptosporidium parvum, Entamoeba dispar, Entamoeba histolytica, Rotavirus**

### REMARKS:

## INCUBATION SCHEME

# Giardia lamblia Antigen (6005)

<b>Dilute patients sample</b>	<b>100 mg sample + 1 ml sample diluent (C)</b>
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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.