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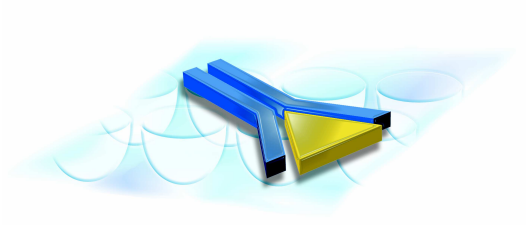

NovaTec EIA

Enzyme immunoassay for the detection of Giardia lamblia

1. Intended use

The Giardia kit is an in vitro diagnosticum intended for the detection of Giardia lamblia antigen in fecal specimens.

2. General

Giardia lamblia is a flagellated enteric protozoan which infects mostly the small intestine after ingestion of Giardia cysts. Its distribution throughout the world makes it an important contributor to chronic debilitating diarrhea and to diarrhea in travelers. The acquisition of the parasite requires oral ingestion of Giardia cysts via fecal contaminated water or food. In the United States, it is the most prevalent infectious agent in waterborne outbreaks of diarrhea. In the developing world, Giardiasis is one of the first enteric pathogens infecting children less than 10 years of age with prevalence rates of 15 - 20 %. Acquisition of Lambliasis occurs mainly in groups with poor fecal-oral hygiene via person-to-person transmission. Such ways of infection occur by children in day care centers, sexually active male homosexuals (up to 19 %) and people in custodial institutions. Many infected young children are symptomatic and spread the disease within their homes and communities. The infection with cysts of the protozoan may be asymptomatic with older children, but they harbor the cysts, excrete them intermittently and remain infectious to other people.
The Giardiasis is characterized as an acute or chronic diarrhea. The incubation period is 3 to 42 days. Clinical manifestations of symptomatic acute infection are sudden onset of watery diarrhea, abdominal cramps and flatulence. The patient expresses feelings of malaise, nausea and anorexia, less frequently vomiting and fever occur; blood, pus and mucus are usually absent.

The diagnosis of Lambliasis in the past was done by stool examination for trophozoites or cysts by microscopy and means of staining. These methods require experienced lab personnel. In addition the investigation must be carried out over a time period since an intermittent excretion of the parasite can occur.
An equivalent method is the new ELISA test for the examination of Giardia lamblia antigen in stool specimen. It shows the same sensitivity as microscopy, needs no experienced personnel for microbiology, is easy and fast and needs no intact organisms (trophozoites or cysts) in stool specimens.

3. Test principle

This test is an enzyme immunoassay (ELISA). On the surface of the microtiter wells, a monoclonal antibody against cell wall proteins (CWP) of Giardia lamblia cysts and trophozoites is bound. Diluted stool samples and controls are pipetted into the wells. A second monoclonal antibody conjugated to horseradish peroxidase is added and then incubated at room temperature.
The simultaneous incubation results in the Giardia lamblia antigen being sandwiched between the solid phase and enzyme-linked antibodies. Unbound POD-conjugate is removed by washing.
Substrate/Chromogen is added to the wells and incubated at room temperature. The enzyme bound in the wells converts the colorless Substrate to a blue color. Addition of Stop Solution converts the color from blue to yellow. The absorption is measured at 450 nm wavelength (optional reference wavelength ≥ 600 nm). The color intensity is directly proportional to the amount of antigen present in the sample.

4. Reagents provided

The reagents in one package are sufficient for 96 determinations.
Each test kit contains:

1 x 12 **Microtiter Strips** with 8 wells each (dividable) in a frame;
coated with monoclonal antibody (mouse) against Giardia lamblia;
in a resealable foil bag
1 x **Parasite Sample Diluent** (100 ml); buffered NaCl solution for sample dilution;
ready to use
1 x **Washing Buffer** (100 ml; 10x conc., brown lid) pH 7.2, contains 0.1 %
Thimerosal

1 x **Positive Control** (1.8 ml); Giardia lamblia antigen from calf stool; ready to use, formalin inactivated
1 **x Enzyme Conjugate** (10 ml); HRP-conjugated mAb (mouse) against Giardia lamblia; dyed green, ready to use, contains 0.01 % Thimerosal
1 x **Substrate/Chromogen** (10 ml); peroxide/TMB; ready to use
1 x **Stop Solution** (6 ml); 1 N sulfuric acid
1 x **Instructions for use**

5. Reagents required but not provided

5.1 Reagents

– Distilled or deionized water

5.2 Accessories

– Test tubes
– Transfer-Pipets (Art. No.: Z 0001)
– Vortex mixer
– Micropipet for volumes of 100 µl and 1 ml
– Microplate washer or multichannel pipet (250 µl)
– Microplate reader (450 nm, optional reference wavelength ≥ 600 nm)
– Absorbent paper

6. Warnings and precautions for the users

The Positive Control contains inactivated antigen of Giardia lamblia. However, the Positive Control as well as the Negative Control and the patient samples should be considered potentially contagious and be treated with the necessary safety precautions.

The Enzyme Conjugate and the Washing Buffer contain Thimerosal. Contact with skin or mucous membranes must be avoided.

Urea peroxide can cause cauterization. Handle with care!

The Stop Solution contains 1 N sulfuric acid. Avoid contact with skin and clothing!

All reagents and materials coming in contact with potential infectious specimens must be treated with disinfectants or autoclaved at 121 °C for at least one hour.

Except of the Parasite Sample Diluent an exchange of individual reagents between kits of different lot numbers is not possible.

Microtiterstrips and reagents should not be used if pouch is damaged or vials are leaking.

7. Storage instructions

All reagents have to be stored at 2 – 8 °C and can be used up to the expiry date printed on the labels. Microbial contamination has to be avoided. A quality warranty cannot be given beyond the kit expiration date.
The diluted Washing Buffer has a shelf life of 4 weeks if stored at 2 – 8 °C.
Allow reagents and Microtiter Strips to get room temperature before use. To avoid moisture within the strips, do not take the strips out of the foil bag before having reached room temperature. The foil bag should be opened with a pair of scissors without detaching the fastener. Return any unused strips to the foil bag, reseal and store them directly at 2 – 8 °C.
The colorless Substrate/Chromogen must be protected from exposure to direct light to avoid deterioration or coloration by autoxidation. If the Substrate/Chromogen turns blue, the reagent should be discarded.

8. Indication of instability or deterioration of reagents

The following criteria may indicate a reagent deterioration:
- a turbidity or a blue coloration of the Substrate/Chromogen prior to its use
- an absorbance value of the Negative Control higher than 0.2
- an absorbance value of the Positive Control lower than 0.8

9. Specimen collection and storage

Stool specimen can be used fresh or frozen. Fresh samples that have not been preserved should be stored at 4 °C and should be tested within 24 h. Storage at 4 °C of a specimen diluted in Parasite Sample Diluent can be prolonged for another 5 days at 2 – 8 °C.
Samples which cannot be tested within this time period should be stored at –20 °C until they are required. Deep freezing does not pose a negative influence on the test results. Repeated thawing and freezing must be avoided.

Samples from MIF enrichment medium are not appropriate for ELISA processing as they may conduct to false results.

10. Test procedure

10.1. Preliminary comments

The test should be used only by experienced laboratory personal. Please refer to guidelines for safety regulations in medical laboratories.The test protocol must be followed strictly.
Bring all reagents and the Microtiter Strips to room temperature before use. Mix the reagents well before use. Reproducibility in any EIA depends on exact pipetting, the observance of incubation times and temperature and the consistency of wash sequences. During the washing steps, take care that all wells are filled with buffer and that the liquid is completely removed from the wells. Do not allow microwells to dry between steps.
Avoid direct sunlight during all incubations. Covering the microtiter plate is recommended.
Except the Washing Buffer, all reagents are ready to use.

10.2. Preparation of the Washing Buffer

1 part of the concentrated Washing Buffer is diluted with 9 parts of distilled water. Crystals in the buffer concentrate can be dissolved in a waterbath at 37 °C. The diluted Washing Buffer has a shelf life of 4 weeks if stored at 2 – 8 °C.

10.3. Preparation of the samples

Using the Parasite Sample Diluent a 1:11 (v/v) dilution of a stool sample is made as followed:
Draw about 100 µl of liquid stool into a Pasteur pipet and suspend in 1 ml of the Parasite Sample Diluent. When using Transfer-Pipet (Art. No. Z 0001) draw just above the second widening (about 100 µl). If the stool is solid, take an equivalent amount (volume of a pea, about 100 mg) with a blade. Homogenize sample by aspiration and ejection with a Transfer-Pipet or by mixing very thoroughly on a vortex-mixer. After allowing a short time to settle (max. 10 minutes) stool suspension can be used directly in the test. If a longer time of settling has passed, the sample should be resuspended before use.

Remark:
Using the Parasite Sample Diluent the stool suspension can be applied to other EIAs for antigen detection in stool. For application in more than three assays a bigger volume of stool suspension should be prepared, for example 2,5 ml Universal Stool Diluent + 0.25 ml stool (≙first mark of Transfer-Pipet).

10.4. First incubation

After a sufficient number of cavities has been placed into the frame, 100 µl of the Positive Control, the Parasite Sample Diluent (Negative Control) and the diluted samples are pipetted into seperate wells. Alternatively: pipet 2 drops with a Transfer-Pipet. 2 drops of Enzyme Conjugate) are added to each well. Mix by gently swirling on tabletop and incubate at room temperature for 60 minutes.

10.5. Washing

Decant or aspirate all wells into a waste container with a disinfectant. Ensure complete removal of the liquid from the wells by tapping the inverted plate onto absorbent paper. Fill 250 µl of prepared Washing Buffer in all wells. Repeat the wash cycle 5 times. Be sure to remove residual washing solution by firmly tapping the inverted microwells on absorbent paper after final washing.

If a microplate washer is used, stool suspension should be discarded manually. During the washing, be sure that the liquid is completely sucked off. After final washing step the inverted microwells should be firmly tapped on absorbent paper.

10.6. Second incubation

Add 2 drops of Substrate/Chromogen into each well. Incubate the plate for 15 min at room temperature in the dark. Following the incubation, the reaction is stopped by adding 1 drop Stop Solution to each well. After careful mixing (soft tapping on the edge of the plate) the absorbance is measured at 450 nm (optional reference wavelength ≥ 600 nm) against an air blank.

Remark:
Highly positive patient samples can cause dark precipitates of the Substrate/Chromogen.

Summary of the test procedure

1. Bring all reagents to room temperature
2. Dilute the Washing Buffer
3. Prepare the Stool Suspension (1:11)
4. Pipet 100 µl (2 drops) of the suspension, the Positive and Negative Control into the microwells
5. Add 2 drops of Enzyme Conjugate; 60 minutes incubation at room temperature
6. Discard the incubate and wash 5 times with 300 µl of Washing Buffer

7. Add 2 drops of Substrate/Chromogen; 15 minutes incubation at room temperature in the dark
8. After addition of 1 drop Stop Solution spectrophotometric determination

11. Analysis

11.1. Quality Control

For the quality control, the Positive and Negative Control must be included in each assay to ensure reagent stability and correct performance of the assay procedure. Controls are intended to monitor for substantial reagent failure. The assay run is correct, if the OD for the Negative Control is below 0.2 and the OD for the positive control is above 0.8. If the Negative Control yields absorbance values >0.2 this may indicate insufficient washing. If the expected control values are not fulfilled please check the following before repeating the test:

- Expiration date of the reagents
- Calibration of the used instruments
- Exact test procedure
- Visual examination of kit components for signs of contamination, deterioration or leakage; substrate solution must not be used if turned blue

If the control data are not fulfilled after repeating, please contact your dealer.

11.2. Calculation of the threshold (cut-off)

The cut-off is determined by addition of 0.15 absorbance units to the measured absorption of the Negative Control.

cut-off = absorbance value of the Negative Control + 0.15

11.3. Interpretation

Samples are considered **positive** if the absorbance value is higher than 10 % over the determined cut-off.

Samples, that have an absorbance value in the area of 10 % above or below the threshold should not be considered as clearly positive or clearly negative. They should be classified as **indeterminate**. It is recommended to test these samples again. As the repeated test with a fresh sample is indeterminate again the sample has to be considered negative.

Samples are considered **negative** if the absorbance value is lower than 10 % under the determined cut-off.

12. Remarks about the test procedure and interpretation

The Giardia assay detects Giardia lamblia antigen in stool specimens. A relation between the absorbance value and the clinical relevance is not given. Assay results should always be interpreted in connection to the clinical diagnosis.

A positive result does not exclude the presence of other pathogens.

A negative result does generally not exclude a Giardia infection. It can due to an intermittent excretion of the parasite. If a reasonable suspicion of an infection exists, a further stool specimen should be investigated.

An indeterminate result can be caused through an unequal dissemination of the parasite within the sample. In this case a second suspension from the same stool sample should be investigated or a further sample should be requested.

13. Clinical results

13.1. Comparative study

The Giardia assay was tested in a clinical study performed with 276 stool specimens. The specimens were taken of german patients who returned from abroad between September 1999 and March 2000. These patients visited the ambulance for infectious diseases and tropical medicine in Munich because of different complaints. All specimens were examined for ova and parasites by means of a direct microscopy for iron-haematoxylin method and SAF-enrichment although each slide was examined of two experienced microscopists for 10 minutes. Aliquots of the fresh stool specimens were frozen at – 20°C immediately after their arrival. After thawing the specimens were examined in the ELISA according to the manufacturer´s instructions. The test showed no cross reactivity with other intestinal parasites. The results are shown in table 1.

Tab. 1: Results of the Giardia Elisa with conventional microscopy

		microscopy	
		positive	negative
Giardia	positive	21	1
	negative	0	254

Sensitivity	100.0 %
Specificity	99.6 %
Positive predictive value	95.5 %
Negative predicitve value	100.0 %

13.2. Cross reactivity-testing with bacteria

Different bacteria were examined, which were used as isolates or as DSM-strains after overnight-culture in BHI-medium containing a suspension of 10^6 germs per ml in duplicate of the test. The extinction is measured at 450 nm. The results are shown in table 2.

Tab. 2: Cross reactivity-testing with bacteria

tested bacteria	origin	result	suspension	supernatant after centrifugation
Acinetobacter lwoffii	DSM 2403	negative	0.004	0.009
Aeromonas hydrophilia anaerogenes	DSM 30022	negative	0.007	0.013
Aeromonas hydrophilia hydrophilia	DSM 30016	negative	0.004	0.009
Citrobacter freundii	DSM 30047	negative	0.007	0.007
Citrobacter freundii	DSM 30039	negative	0.005	0.007
Enterobacter cloacae	DSM 30054	negative	0.003	0.007
Enterococcus faecalis	DSM 2570	negative	0.002	0.002
Enterococcus faecium	DSM 20477	negative	0.001	0.001
Escherichia coli	Isolate	negative	0.001	0.008
Escherichia coli	Isolate	negative	0.006	0.011
Escherichia coli	Isolate	negative	0.005	0.011
Escherichia hermannii	DSM 4560	negative	0.006	0.006
Lactococcus lactis	DSM 20481	negative	0.007	0.005
Listeria innocua	SLCC 5639	negative	0.004	0.008
Proteus mirabilis	DSM 788	negative	0.002	0.003
Proteus mirabilis	DSM 4479	negative	0.001	0.001
Proteus vulgaris	DSM 30119	negative	0.005	0.010
Providencia stuartii	DSM 6676	negative	0.007	0.011
Pseudomonas aerruginosa	DSM 939	negative	0.009	0.009
Pseudomonas fluorescens	DSM 4358	negative	0.004	0.009
Pseudomonas fluorescens	DSM 50124	negative	0.006	0.007
Pseudomonasputida	DSM 291	negative	0.005	0.011
Salmonella Agona	Isolate	negative	0.001	0.006
Salmonella Cholerasuis	DSM 4224	negative	0.004	0.002
Salmonella Infantis	Isolate	negative	0.006	0.014
Salmonella Ohio	Isolate	negative	0.006	0.012
Salmonella Typhimurium	Isolate	negative	0.004	0.012
Serratia proteamaculans	DSM 4487	negative	0.007	0.007
Shigella flexneri	DSM 4782	negative	0.009	0.009

Shigella sonnei	DSM 5570	negative	0.008	0.007
Staphylococcus aureus	DSM 20372	negative	0.005	0.003
Streptococcus agalactiae	Isolate	negative	0.038	0.006
Streptococcus dysgalactiae	Isolate	negative	0.005	0.010
Streptococcus uberis	Isolate	negative	0.008	0.013
Escherichia coli (O157:H-)	Isolate	negative	0.005	0.010
Escherichia coli (O116:H21)	Isolate	negative	0.005	0.009
Escherichia coli (O111:H-)	Isolate	negative	0.007	0.010
Escherichia coli (O26:H8)	Isolate	negative	0.004	0.008
Negative Control BHI			0.004	0.008
Positive Control test kit			1.796	1.635
cut-off			0.146	0.142

13.3. Cross reactivity-testing with worm-ova and other parasites

Different microscopically determinded stool specimens were examined, which were used in a 1:11 dilution in the ELISA. The results are shown in table 3.

Tab. 3: Cross reactivity-testing with worm-ova and other parasites

Microscopical result	Giardia (cut-off negative control + 0.150)
Ova of Schistosoma mansoni	negative
Ova of Taenia solium	negative
Ova of Trichuris trichiura	negative
Ova of Ascaris lumbricoides	negative
Ova of Enterobius vermicularis	negative
Ova of Hymenolepis nana	negative
Ova of Ankylostoma duodenale	negative
Entamoeba coli cysts	negative
Entamoeba hartmanni cysts	negative
Iodamoeba bütschlii cysts	negative
Blastocystis hominis cysts	negative
Cryptosporidien-cysts	negative

13.4. Precision

The Intra-assay variation was assayed in 24 fold determination of the Positive Control (cysts-suspension) as well as in a medium- (Pr. 32) and in a low-titrated (Pr. 754) stool specimen and in the Negative Control (dilution buffer USP). The results are shown in table 4.

Tab. 4: Intra-assay reproducibility

	PK	Pr. 32	Pr. 754	USP
MW (OD)	2.343	0.987	0.533	0.045
SD	0.138	0.068	0.028	0.002
VK %	5.9	6.9	5.3	4.4

The Inter-assay variation of the Giardia Test was assayed through a 4 fold determination on 4 days and 4 different test kits of one batch with the same specimens, which also were used in the Intra-assay test. The results are shown in table 5.

Tab. 5: Inter-assay reproducibility

	PK	Pr. 32	Pr. 754	USP
MW (OD)	1.988	1.021	0.592	0.052
SD	0.102	0.037	0.043	0.006
VK %	5.13	3.62	7.26	11.54

Appendix

Literature

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GIA0160engl08062005-CR