Giardia lamblia ELISA Kit

Cat. No.:DEIA7752
Pkg.Size:96T

Intended use

The Giardia lamblia ELISA Kit is intended for the qualitative determination of Giardia lamblia antigensin faeces.

General Description

Giardia lamblia (Lamblia intestinalis) is a flagellated parasite affecting the intestinal tissue of animal and man and is found in all parts of the world, especially in the tropics. As trophozoite the parasite is highly motile. The cysts will be spread in water and this allows the transfer from host to host, e.g. with contaminated food, or by sexual transmission.
The disease may manifest itself as an acute, chronic or as an asymptomatic infection. The frequency of infection varies greatly with the region and is dependent on local living conditions. Local epidemics are mostly related to the quality of water supply (contamination hazard). Infection often proceeds without symptoms or very mild, parasites may be eliminated spontaneously. In cases of persistent infection patients show chronical recurrent diarrhoea. Mainly children are affected and risk groups of immunodeficient persons.

Principle Of The Test

The qualitative immunoenzymatic determination of Giardia lamblia antigens is based on the ELISA (Enzyme-linked Immunosorbent Assay) technique.
Microtiter strip wells are precoated with polyclonal anti-Giardia lamblia antibodies to Giardia lamblia cysts and trophozoites. Diluted patient specimen (stool), and ready to use controls are added to these wells and during the first incubation, Giardia antigens present in the stool supernatant are captured. After washing the wells to remove all unbound sample and control a horseradish peroxidase labelled anti-Giardia antibody is added which binds to the captured antigen. After incubation and washing off unbound enzyme the antigen-conjugate-complex is visualized with TMB substrate which gives a blue reaction product, the intensity is proportional to the amount of Giardia lamblia antigens in the patient specimen. Sulphuric acid is added to each well to stop the reaction and turns the blue colour to yellow. Absorbance at 450 nm is read using an ELISA reader.

Reagents And Materials Provided

A. Reagents supplied
1. Giardia lamblia Coated Wells (antigen): 12 breakapart 8-well snap-off strips coated with polyclonal anti-Giardia lamblia antibodies; vacuum sealed, in resealable aluminium foil.
2. Sample Diluent*: 1 bottle containing 100 ml of ready to use buffer for sample dilution; pH 8.4 ± 0.1;
3. Washing Solution (10x conc.)*: 1 bottle containing 100 ml of a 10-fold concentrated buffer for washing the wells; pH 7.4±0.1;
4. Stop Solution: 1 bottle containing 15 ml sulphuric acid, 0.25 mol/l; ready to use;
5. Anti-Giardia lamblia Conjugate**: 1 bottle containing 12 ml of peroxidase labelled anti-Giardia lamblia antibodies; coloured green; ready to use;
6. TMB Substrate Solution***: 1 bottle containing 15 ml 3,3',5,5'-Tetramethylbenzidine (TMB); ready to use;
7. Giardia lamblia Antigen Positive Control**: 1 bottle containing 2 ml; ready to use; coloured blue;
8. Giardia lamblia Antigen Negative Control*: 1 bottle containing 2 ml; ready to use; coloured blue;
* contains 0.01 % Thimerosal

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** contains 1.0 % Kathon
*** contains 0.008 % Kathon
B. Materials supplied
1. 1 Strip holder
2. 2 Cover foils
3. 1 Test protocol
4. 1 distribution and identification plan

Materials Required But Not Supplied

1. ELISA microwell plate reader, equipped for the measurement of absorbance at 450/620 nm
2. Manual or automatic equipment for rinsing wells
3. Pipettes to deliver volumes between 10 and 1000 µl
4. Vortex tube mixer
5. Deionised or (freshly) distilled water
6. Disposable tubes
7. Timer

Storage

The reagents are stable up to the expiry date stated on the label when stored at 2...8℃. After first opening the reagents are stable for another 2 months if stored at +2 to +8℃.

Specimen Collection And Handling

The test is intended for the detection of Gardia lamblia in diluted stool specimen. Either fresh or frozen specimen may be used in this test. If samples are stored frozen, mix thawed samples well before dilution. If the assay is performed within 24 hours after sample collection, the specimen should be kept at 2...8℃; otherwise they should be aliquoted and stored deep-frozen (-20 to -70℃). Avoid repeated freezing and thawing.
1. Sample Dilution
Before assaying, all fresh or frozen samples should be diluted 1+10 with Sample Diluent. Dispense 100 mg or 100 µl of faecal sample and 1 ml of Sample Diluent into tubes to obtain a 1+10 dilution and thoroughly mix with a Vortex. If the standing time of a diluted sample is longer than 10 min it has to be mixed again thoroughly before starting the test run. Do not use concentrated stool samples. The assay will not give accurate results on a concentrated sample. Positive and negative controls are ready to use and must not be diluted.

Reagent Preparation

1. Coated snap-off Strips
The ready to use breakapart snap-off strips are coated with polyclonal antibodies (rabbit) against Giardia lamblia. Store at 2...8℃. The strips are vacuum sealed. Immediately after removal of strips, the remaining strips should be resealed in the aluminium foil along with the desiccant supplied and stored at 2...8℃.
2. Anti-Giardia lamblia Conjugate
The bottle contains 12 ml of a solution of horseradish peroxidase labelled antibodies against Giardia lamblia cysts and trophozoites, buffer, stabilizers, preservatives and an inert green dye. The solution is ready to use. Store at 2...8℃.
3. Controls
The bottles labelled with Positive and Negative Control contain a ready to use control solution. It has to be stored at 2...8℃.
4. Sample Diluent

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The bottle contains 100 ml Tris buffer and preservatives. It is used for the dilution of the patient specimen. This ready to use solution has to be stored at 2...8℃.
5. Washing Solution (10x concentrated)
The bottle contains 100 ml of a concentrated buffer. Dilute washing solution 1+9; e.g. 10 ml washing solution + 90 ml fresh and germ free redistilled water. The diluted buffer is stable for one month if stored at 2…8℃.
6. TMB Substrate Solution
The bottle contains 15 ml of a tetramethylbenzidine/hydrogen peroxide system. The reagent is ready to use and has to be stored at
2...8℃, away from the light. The solution should be colourless or could have a slight blue tinge. If the substrate turns into blue, it may have become contaminated and should be thrown away.
7. Stop Solution
The bottle contains 15 ml 0.25 M sulphuric acid solution (R 36/38, S 26). This ready to use solution has to be stored at 2...8℃.

Assay Steps

1. Test Preparation
Please read the test protocol carefully before performing the assay. Result reliability depends on strict adherence to the test protocol as described. Prior to commencing the assay, the distribution and identification plan for all specimens and controls should be carefully established on the result sheet supplied in the kit. Select the required number of microtiter strips or wells and insert them into the holder.
Please allocate at least:
1 well (e.g. A1) for the substrate blank,
2 wells (e.g. B1+C1) for the negative control and
1 well (e.g. D1) for the positive control.
It is recommended to determine controls and patient samples in duplicate. Perform all assay steps in the order given and without any appreciable delays between the steps. A clean, disposable tip should be used for dispensing each control and sample.
1. Dispense 2 drops (or 75 µl) ready to use controls and 100µl diluted stool samples (supernatant) into their respective wells. Leave well A1 for substrate blank.
2. Cover wells with the foil supplied in the kit.
3. Incubate for 30 ±1 min at room temperature. (20…25℃) without shaking.
4. When incubation has been completed, remove the foil, aspirate the content of the wells and wash each well five times with 400µl of Washing Solution. Avoid overflows from the reaction wells. The soak time between each wash cycle should be >5 sec. At the end carefully remove remaining fluid by tapping strips on tissue paper prior to the next step!
Note: Washing is critical! Insufficient washing results in poor precision and falsely elevated absorbance values.
5. Dispense 2 drops (or 75 µl) of Anti-Giardia lamblia Conjugate into all wells except for the blank well (e.g. A1). Cover with foil.
6. Incubate for 30 ±1 min at room temperature. (20…25℃) without shaking.
7. Repeat step 4.
8. Dispense 2 drops (or 75 µl) of TMB Substrate Solution into all wells.
9. Incubate for 10 ±1 min at room temperature (20…25℃) in the dark.
10. Dispense 2 drops (or 75 µl) of Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate Solution. Any blue colour developed during the incubation turns into yellow.
Note: Highly positive patient samples can cause dark precipitates of the chromogen! These precipitates have an influence when reading the optical density. A further dilution of the supernatant with Sample Diluent is recommended; for example 1+1. After performing the test multiply the results in OD by 2.
a. Measure the absorbance of the specimen at 450/620 nm within 30 min after addition of the Stop Solution.

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2. Measurement
Adjust the ELISA Microwell Plate Reader to zero using the substrate blank in well A1.
If - due to technical reasons - the ELISA reader cannot be adjusted to zero using the substrate blank in well A1, subtract the absorbance value of well A1 from all other absorbance values measured in order to obtain reliable results!
Measure the absorbance of all wells at 450 nm and record the absorbance values for each control and patient sample in the distribution and identification plan.
Dual wavelength reading using 620 nm as reference wavelength is recommended. Where applicable calculate the mean absorbance values of all duplicates.

Interpretation of Results

1. Run Validation Criteria
In order for an assay to be considered valid, the following criteria must be met:
Negative control in B1 and C1: Absorbance value ≤ 0.200.
Positive control in D1: Absorbance value ≥ 0.800
2. Calculation of Results
The cut-off is calculated by addition of 0.20 absorbance units to the measured absorption of the mean value of the two negative control determinations.
Example: 0.12 OD neg. control + 0.14 OD neg. control = 0.26 ÷ 2 = 0.13
Cut-off = absorbance mean value of the negative control + 0.20
Cut-off = 0.13 + 0.20 = 0.33
Samples are considered POSITIVE if the absorbance value is higher than the cut-off. Samples are considered NEGATIVE if the absorbance value is lower than the cut-off.

Sensitivity

The diagnostic sensitivity is defined as the probability of the assay of scoring positive in the presence of the specific analyte. 40 known positive samples were tested with this assay resulting in a sensitivity of 97.5% (39/40).

Specificity

The diagnostic specificity is defined as the probability of the assay of scoring negative in the absence of the specific analyte. 369 known negative samples were tested with this assay resulting in a specificity of 99.5% (367/369).

Reproducibility

Intra-assay: 6.41%-10.9%
Inter-assay: 4.64%-10.9%

Precautions

1. In compliance with article 1 paragraph 2b European directive 98/79/EC the use of the in vitro diagnostic medical devices is intended by the manufacturer to secure suitability, performances and safety of the product. Therefore the test procedure, the information, the precautions and warnings in the instructions for use have to be strictly followed. The use of the testkits with analyzers and similar equipment has to be validated. Any change in design, composition and test procedure as well as for any use in combination with other products not approved by the manufacturer is not authorized; the user himself is responsible for such changes. The manufacturer is not liable for false results and incidents for these reasons. The manufacturer is not liable for any results by visual analysis of the patient samples.
2. All components of human origin used for the production of these reagents have been tested for anti-HIV antibodies, anti-HCV

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antibodies and HBsAg and have been found to be non-reactive. Nevertheless, all materials should still be regarded and handled as potentially infectious.
3. Do not interchange reagents or strips of different production lots.
4. No reagents of other manufacturers should be used along with reagents of this test kit.
5. Do not use reagents after expiry date stated on the label.
6. Use only clean pipette tips, dispensers, and lab ware.
7. Do not interchange screw caps of reagent vials to avoid cross-contamination.
8. Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination.
9. After first opening and subsequent storage check conjugate and control vials for microbial contamination prior to further use.
10. To avoid cross-contamination and falsely elevated results pipette patient samples and dispense conjugate without splashing accurately to the bottom of wells.

Limitations

The lower detection limit of Giardia lamblia antigen was determined by titration of faecal samples spiked with Giardia lamblia cysts and trophozoites from culture. The lower detection limit for Giardia lamblia was determined 5 x 103 cysts and 2,5x104 trophozoites per ml of diluted faecal sample.
There is no correlation between the measured absorbance and the severity of the disease and the absorbances of samples shouldn't be correlated to the absorbances 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 resuspension may produce false negative results. Bacterial contamination or repeated freeze-thaw cycles of the specimen may affect the absorbance values.
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. Diagnosis of an infectious disease should not be established on the basis of a single test result. A precise diagnosis should take into consideration clinical history, symptomatology as well as serological data.

REFERENCES

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

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