Giardia lamblia ELISA

Enzyme immunoassay for the qualitative determination of Giardia specific antigens in faecal specimens.

REF  EU56001

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1. INTENDED USE

Enzyme immunoassay for the qualitative determination of Giardia specific antigens in faecal specimens.

2. SUMMARY AND EXPLANATION

Giardiasis is a common cause of gastroenteritis in humans and known to affect at least 200 million people worldwide with 2 % of adults and 6-8 % of children in developing countries getting infected each year. Nearly 33 % of the population in developing countries has contracted the disease at least once in their life. In the USA and other industrialized countries it is also by far the most common intestinal parasitic disease found in humans.

Giardiasis is caused by a flagellated protozoan parasite, Giardia lamblia. The life cycle of the parasite begins with ingestion of cysts by the host. Following excystation, active trophozoites emerge which colonize the lumen of the small intestine to feed. After the feeding stage, the parasites replicate asexually through longitudinal binary fission. During their passage through the digestive system, some parasites are converted to cysts. Both cysts and trophozoites are then excreted with the faeces but only the hardy, resistant cysts can persist for weeks to months outside the host, in soils, on surfaces or in stagnant water systems like ponds, swimming pools, water reservoirs.

Humans usually become infected by the parasite in several ways:
- Transmission by waterborne sources: swallowing of dormant cysts present in contaminated water or food.
- Transmission by faecal-oral route in situations with poor hygienic practice, e.g. in day-care centres.
- Close contact with infected persons.
- Since domestic animals like cats, dogs, cattle, birds but also wild mammals (e.g. deer, beaver) act as reservoir hosts for Giardia, a zoonotic transmission is also a possibility.

Upon infection, trophozoites colonizing the small intestine induce inflammation, apoptosis of intestinal epithelial cells, morphological changes to the microvilli and villar atrophy, causing problems with the small intestine's absorption system (failure to absorb fat, lactose, vitamin A and B 12). Parasites do not enter the bloodstream however and do not spread to other parts of the gastrointestinal tract.

Symptoms of acute giardiasis typically set in one to two weeks after exposure to the parasite and may include diarrhea, hematuria, flatulence, nausea, greasy stool, stomach and abdominal cramps, intestinal malabsorption, dehydration and weight loss. Up to 50-60 % of cases seem to be asymptomatic.

The disease normally resolves by itself after a mere six weeks if left untreated but may persist for longer periods in immunocompromised patients. Severe giardiasis might delay physical and mental growth and cause malnutrition in children. In some people, particularly those with a lack of IgA antibodies, recurring infections can develop into a chronic state of giardiasis.

Diagnosis of giardiasis is not always straightforward due to a lack of symptoms in many cases. Useful diagnostic methods are:

Invasive techniques:

• Duodenal biopsy to detect presence of trophozoites.
• String test or entero-test: a gelatin capsule with a siring attached is swallowed by the patient. When it passes into the small intestine, trophozoites get stuck to the string. The string is then withdrawn from the patient and examined microscopically for parasites.
Non-invasive techniques:

- Stool microscopy: examination of stool samples for presence of trophozoites or cysts. Because parasites are shed intermittently, multiple stool collections are necessary. This method relies on experienced lab technicians for a correct diagnosis because infections are often difficult to demonstrate.
- ELISA to detect Giardia antigens in stool samples. Faecal immunoassays offer comparable or even better sensitivity and specificity to microscopic examination and are fairly simple to perform.
- Molecular testing (PGR).

Of the non-invasive techniques, microscopic examination of stools has been the most common but this method relies on the expertise of the technician. Because of the historically low proficiency of correct microscopic examinations and intermittent excretion of organisms, alternative diagnostic methods have been investigated and it has been shown that using the ELISA method, comparable sensitivity can be achieved.

3. PRINCIPLE OF PROCEDURE

During the first incubation, Giardia specific antigen present in the stool specimens are captured by antibodies attached to the microwells. The wells are incubated and washed before anti-Giardia antibodies conjugated to peroxidase are added. The enzyme conjugate will "sandwich" any antigen bound to the wells. After washings to remove unbound enzyme, a chromogen is added which develops a blue color in the presence of the enzyme complex. The stop solution ends the reaction and turns the blue color to yellow. If no antigen is captured, or if there is an insufficient level of antigen, no colored reaction will take place.

4. REAGENTS

1. **MTP**
   - Microtiterplate
   - 12 x 8-well strips coated with anti-Giardia antibodies.

2. **CONTROL +**
   - Positive Control
   - 1 vial, containing 2 mL of a diluted Giardia positive formalinized stool supernatant.

3. **CONTROL -**
   - Negative Control
   - 1 vial, containing 2 mL of a Giardia negative formalinized stool supernatant.

4. **ENZCONJ**
   - Enzyme Conjugate
   - 1 vial, 11 mL of peroxidase labeled anti-Giardia antibodies with Thimerosal.

5. **SAMPLEDIL**
   - Sample Diluent Buffer
   - 1 vial, containing 60 mL of a buffered solution with detergent and Thimerosal.

6. **WASHBUF CONC**
   - Wash Buffer
   - 1 vial containing 50 mL 20 x concentrated buffer with detergent and Thimerosal.

7. **TMB SUBS**
   - TMB Substrate Solution
   - 1 vial, containing 11 mL chromogen/substrate solution.

8. **STOP**
   - TMB Stop Solution
   - 1 vial, containing 11 mL (Phosphoric acid 5 %)
5. MATERIALS REQUIRED BUT NOT SUPPLIED
1. Transfer Pipettes
2. Graduated Cylinder
3. Reagent grade (DI) water
4. ELISA plate reader with 450 and 620-650 nm filters
5. Vortex mixer
6. Wash bottle, automated or semi-automated microtiter plate washing system
7. Paper towels, pipette tips and timer

6. WARNINGS AND PRECAUTIONS FOR USERS
1. For In Vitro Diagnostic Use. For professional use only.
2. Before starting the assay, read the instructions completely and carefully. Use the valid version of the package insert provided with the kit. Be sure that everything is understood.
3. In case of severe damage of the kit package please contact IBL or your supplier in written form, latest one week after receiving the kit. Do not use damaged components in test runs, but keep safe for complaint related issues.
4. Follow good laboratory practice and safety guidelines. Wear lab coats, disposable latex gloves and protective glasses where necessary.
5. Reagents of this kit containing hazardous material may cause eye and skin irritations. See MATERIALS SUPPLIED and labels for details. Material Safety Data Sheets for this product are available on the IBL-Homepage or upon request directly from IBL.
6. Chemicals and prepared or used reagents have to be treated as hazardous waste according to national biohazard and safety guidelines or regulations.
7. The cleaning staff should be guided by the professionals regarding potential hazards and handling.
8. Do not use solutions if they precipitate or become cloudy. Exception: Wash concentrate may precipitate during refrigerated storage, but will dissolve upon warming.
9. Do not add azides to the samples or any of the reagents. Controls and some reagents contain Thimerosal as a preservative.
10. Treat all reagents and samples as potentially infectious materials. Use care to prevent aerosols and decontaminate any spills of samples.
11. Stop solution is a 5 % solution of phosphoric acid in water. If spilled on the skin, wash with copious amounts of water. If acid gets into the eyes, wash with copious amounts of water and seek medical attention.
12. Persons who are color blind or visually impaired may not be able to read the test visually and should use spectrophotometric readings to interpret results.

7. STORAGE CONDITIONS
Reagents, strips and bottled components: Store between 2-8 °C.
Bottle containing diluted wash buffer may be stored at room temperature.

8. COLLECTION OF STOOL (FAECES)
No modification of collection techniques used for standard microscopic O&P is needed. Stool samples may be used as unpreserved or frozen, or in preservation media of 10 % formalin, SAF or MF.
Unpreserved samples should be kept at 2-8 °C and tested within 24 hours of collection. Samples that cannot be tested within this time should be frozen at -20 °C or lower until used. Freezing does not adversely affect the test.
Formalized, SAF and MF preserved samples may be kept at room temperature (15-25 °C) and tested within 18 months of collection. DO NOT freeze preserved samples. All dilutions of unpreserved stools must be made with the Dilution Buffer provided.
9 PREPARATION OF SAMPLE

Fresh/Frozen Stools

Thaw sample if needed. Prepare a 1:4 dilution in tubes using 0.3 mL of Dilution Buffer and one swab of fecal specimen (approximately 0.1 g). Coat swab with specimen and transfer into the Dilution Buffer, expressing as much liquid as possible and mix well. For watery specimens, add 0.1 mL of sample to 0.3 mL Dilution Buffer in tubes. Special designed faecal preparation tubes can be used for sample preparation. For automatic ELISA devices it is advised to centrifuge the samples before use.

Preserved Stools (Formalin, SAF and MF)

Mix contents thoroughly inside collection container. No further processing is required.

10 ASSAY PROCEDURE

General remarks:

1. Use a separate disposable tip for each sample transfer to avoid cross-contamination.
2. All reagents must be allowed to come to room temperature before use. All reagents must be mixed without foaming.
3. Once the assay has been started, all steps should be completed without interruption.
4. Any improper handling of samples or modification of the test procedure may influence the results. The indicated pipetting volumes, incubation times, temperatures and pretreatment steps have to be performed strictly according to the instructions. Use calibrated pipettes and devices only.
5. It is advised to determine samples in duplicate to be able to identify potential pipetting errors.
6. Use a pipetting scheme to verify an appropriate plate layout.
7. Microtiter plate washing is important. Improperly washed wells will give erroneous results. It is recommended to use a multichannel pipette or an automatic microtiter plate washing system. Do not allow the wells to dry between incubations. Do not scratch coated wells during rinsing and aspiration.
8. Rinse and fill all reagents with care. While rinsing, check that all wells are filled precisely with Wash Buffer, and that there are no residues in the wells

Reconstitution of the Reagents:

Wash Buffer - Remove cap and add contents of one bottle of Wash Concentrate to a bottle containing 950 mL of DI water. Swirl to mix.

CAUTION: Crystals may form when the concentrated washing solution is stored at 2-8 °C These crystals can easily be dissolved when bringing the vials to room temperature or by placing them in a water bath at 37 °C.

Assay Procedure:

1. Break off the required number of wells needed (number of samples plus 2 for controls) and place in holder.
2. Add 100 µL of negative control to well # 1 and 100 µL of positive control to well # 2.*
3. Add 50 µL of dilution buffer to each sample well. DO NOT add dilution buffer to control wells.
4. Add 50 µL of sample to each well with dilution buffer.
5. Incubate for 60 minutes at room temperature (15-25 °C), then wash**. After last wash slap the wells out on a clean absorbent towel to remove left over wash buffer.
6. Add 100 µL of Enzyme Conjugate to each well.
7. Incubate for 30 minutes at room temperature (15-25 °C), then wash**. After last wash slap the wells out on a clean absorbent towel to remove left over wash buffer.
8. Add 100 µL of Chromogen to each well.
9. Incubate 10 minutes at room temperature (15-25 °C). For automatic ELISA devices incubate 8 minutes at room temperature.
10. Add 100 µL of Stop Solution to each well. Mix wells by gently tapping the side of the strip holder with index finger.
11. Read results visually or at 450/620-650 nm.
Controls must be included each time the kit is run.

Washings consist of vigorously filling each well to overflowing and decanting contents seven separate times. For automatic ELISA devices the washing consists of seven wash steps using a volume of 400 µL.

Only one set of controls is required per run.
Read results within 4 hours from addition of Stop Solution.
All incubations are done at room temperature (15-25 °C).

11 RESULTS

Interpretation of Results - Visual

Reactive: Any sample well that is obviously more yellow than the negative control well.
Non-reactive: Any sample well that is not obviously more yellow than the negative control well.

NOTE: The negative control, as well as some samples, may show some slight color. A sample well must be obviously darker than the negative control well to be called a positive result. Please refer to the enclosed visual read card for color comparisons.

Interpretation of Results - ELISA Reader

Read all wells at 450/620-650 nm.
Reactive: Absorbance reading of 0.08 OD units and above indicates the sample contains Giardia antigen.
Non-reactive: Absorbance reading less than 0.08 OD units indicates the sample does not contain detectable levels of Giardia antigen.

Expected Values

The results themselves should not be the only reason for any therapeutical consequences. They have to be correlated to other clinical observations and diagnostic tests.

Normal healthy individuals should be free of Giardia and should test negative. A positive reaction indicates that the patient is shedding detectable amounts of Giardia antigen. Certain populations, such as homosexual men and children in day care settings, have shown higher rates of infection with Giardia than the normal population. Please refer to the Summary section for references.

12 LIMITATION OF PROCEDURE

Test results should be used as an aid in diagnosis and should not be interpreted as diagnostic by themselves.
DO NOT concentrate stool samples. Assay will not give accurate results on a concentrated sample.
A negative result can occur from an antigen level lower than the detection limits of this assay. Multiple samples over time may be indicated for those patients that are suspected of being positive for Giardia.

13 QUALITY CONTROL

The test results are only valid if the test has been performed following the instructions. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or comparable standards/laws. User and/or laboratory must have a validated system to get diagnosis according to GLP. All kit controls must be found within the acceptable ranges as stated on the labels and the QC certificate. If the criteria are not met, the run is not valid and should be repeated. Each laboratory should use known samples as further controls.

The use of a positive and negative control allows easy validation of kit stability. For a valid test, the positive control must have an absorbance of at least 0.5 OD units and the negative control must be less than 0.08 OD units. Should the value fall outside these limits, the kit should not be used.
14  REPRODUCIBILITY

The intra-assay (well to well) CV was calculated using 4 positive and 4 negative samples assayed 24 times in a single run. The mean CV was 3.67 % with the highest being 6.18 %.

The inter-assay (run to run) CV was calculated using 4 positive and 4 negative samples assayed on three separate days. The mean CV was 4.08 % with the highest being 11.61 %.

15  CROSS-REACTIVITY

No cross-reactions were seen with the following organisms:
Entamoeba hartmanni, Endolimax nana, Entamoeba histolytica/dispar, Entamoeba coli, Blastocystis hominis, Dientamoeba fragilis, Chilomastix mesnili, Strongyloides stercoralis, Cryptosporidium, Ascaris lumbricoides, Enterobius vermicularis, Diphyllobothrium species, Hymenolepis nana, Clonorchis sinensis, Enteromonas hominis, Trichuris trichiura, Iodamoeba buetschlii, Hookworm, Schistosoma mansoni, rotavirus, Taenia eggs, Fasciola eggs, Isospora belli, Entamoeba polecki, adenovirus, & 33 bacterial species (list available on request).

16  TROUBLESHOOTING

Problem: Negative control has substantial color development.
Correction: Washings were insufficient. Repeat test with more vigorous washings.

17  BIBLIOGRAPHY

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Symbols of the kit components see MATERIALS SUPPLIED.
Die Symbole der Komponenten sind im Kapitel KOMPONENTEN DES KITS beschrieben.
Voir MATERIEL FOURNI pour les symbôles des composants du kit.
Símbolos de los componentes del juego de reactivos, vea MATERIALES SUMINISTRADOS.
Para símbolos dos componentes do kit ver MATERIAIS FORNECIDOS.
Per i simboli dei componenti del kit si veda COMPONENTI DEL KIT.
Για τα σύμβολα των συστατικών του κιτ συμβουλεύετε το ΠΑΡΕΧΟΜΕΝΑ ΥΛΙΚΑ.

COMPLAINTS: Complaints may be submitted initially written or vocal. Subsequently they need to be filed including the test performance and results in writing in case of analytical reasons.

WARRANTY: The product is warranted to be free from material defects within the specific shelf life and to comply with product specifications delivered with the product. The product must be used according to the Intended use, all instructions given in the instructions for use and within the product specific shelf life. Any modification of the test procedure or exchange or mixing of components of different lots could negatively affect the results. These cases invalidate any claim for replacement.

LIMITATION OF LIABILITY: IN ALL CIRCUMSTANCES THE EXTENT OF MANUFACTURER’S LIABILITY IS LIMITED TO THE PURCHASE PRICE OF THE KIT(S) IN QUESTION. IN NO EVENT SHALL MANUFACTURER BE LIABLE FOR ANY INCIDENTAL OR CONSEQUENTIAL DAMAGES, INCLUDING DAMAGES FOR LOST PROFITS, LOST SALES, INJURY TO PERSON OR PROPERTY OR ANY OTHER INCIDENTAL OR CONSEQUENTIAL LOSS.

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