Giardia lamblia ELISA

REF 610001

apDia *Giardia lamblia* ELISA is an *In Vitro* Diagnostic (IVD) immunoassay for the qualitative determination of *Giardia* specific antigens in faecal specimens.

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 B12). 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 string 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 (PCR).

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. It has been shown that by using the ELISA method, a comparable sensitivity can be achieved.

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.

REAGENTS

1. Coated Microtiterstrips - MTP
Microwells containing anti-*Giardia* monoclonal antibodies
96 test wells in a test strip holder.
2. Negative control - CTLNEG
1 vial, containing 2 ml of Specimen diluent
3. Positive control - CTLPOS
1 vial, containing 2 ml of a diluted *Giardia*-positive formalinized stool supernatant.
4. Wash Buffer - WASH 20x
1 bottle, containing 50 ml of a 20x concentrated buffer with detergent and thimerosal.
5. Enzyme conjugate - CONJ
1 bottle, containing 11 ml of peroxidase-labeled anti-*Giardia* polyclonal antibodies with preservative.
6. Specimen diluent - DIL
1 bottle, containing 60 ml of a buffered solution with detergent and thimerosal.
7. Chromogen/ Substrate - CHROM
1 bottle, containing 11 ml chromogen/substrate solution.
8. Stop solution - STOP
1 vial, containing 11 ml of phosphoric acid 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

WARNINGS AND PRECAUTIONS FOR USERS

For *In Vitro* Diagnostic Use
1. Do not use solutions if they precipitate or become cloudy.
Exception: Wash concentrate may precipitate during refrigerated storage, but will dissolve upon warming.
2. Do not add azides to the samples or any of the reagents. Controls and some reagents contain thimerosal as a preservative.
3. Treat all reagents and samples as potentially infectious materials. Use care to prevent aerosols and decontaminate any spills of samples.
4. 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.
5. 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.

STORAGE CONDITIONS

Reagents, strips and bottled components: store between 2 - 8ºC.
Bottle containing diluted wash buffer may be stored at room temperature.

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 - 8º C and tested within 24 hours of collection. Samples that cannot be tested within this time should be frozen at -20º C or lower until used. Freezing does not adversely affect the test.
Formalized, SAF and MF preserved samples may be kept at room temperature (15-25º C) or at 2-8º C and tested within 18 months of collection.
DO NOT freeze preserved samples.
All dilutions of unpreserved stools must be made with the provided Specimen Diluent.

PREPARATION OF SAMPLE

Fresh/Frozen Stools
Thaw sample if needed. Prepare a 1:4 dilution in tubes using 0.3 ml of Specimen Diluent and one swab of faecal specimen (approximately 0.1 g). Coat swab with specimen and transfer into the Specimen Diluent, expressing as much liquid as possible and mix well. For watery specimens, add 0.1 ml of sample to 0.3 ml Specimen Diluent 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.

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.

Reconstitution of the Reagents:
Wash Buffer - Remove cap and add contents of one bottle of the concentrated washing buffer 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 Specimen Diluent to each sample well. DO NOT add Specimen Diluent to control wells.
4. Add 50 µl of sample to each well with Specimen Diluent.
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 remaining 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 remaining 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).

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.

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
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.

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*.

QUALITY CONTROL

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.

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%

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).

TROUBLESHOOTING

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

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In vitro diagnostic kit

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