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User's Manual

Giardia lamblia ELISA

Immunoassay for the qualitative determination of *Giardia* specific antigens in faecal specimens

CE

IVD

REF DE610001

Σ 96 wells

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SUMMARY AND EXPLANATION

Giardia lamblia is the protozoan parasite responsible for the disease giardiasis. Symptoms of acute giardiasis include diarrhea, nausea, weight loss, malabsorption, abdominal cramps, flatulence and anemia.[1] The disease may manifest itself as an acute, chronic or as an asymptomatic infection. Giardiasis is the most prevalent parasitic disease in the United States and is responsible for an estimated 100 million mild infections and 1 million severe infections each year.[9]
The mode of transmission of *Giardia* is through faecal-oral ingestion of cysts. Epidemics of giardiasis have been documented in day care centres and by drinking contaminated water.[1,2] Day care centres may be directly or indirectly responsible for 45% of diagnosed *Giardia* infections in the United States.[4] One study found 54% of the children at a day care centre were infected.[1]
Another important source of *Giardia* infection is among homosexual men. Prevalence rates of 5 to 19% for this population have been reported.[8]
Diagnosis of giardiasis has been done through a number of invasive and non-invasive techniques. Of the non-invasive techniques, microscopic examination of stools has been the most common. However, this method relies on an experienced technician and subsequent observation of intact organisms. Because of the historically low proficiency of correct microscopic examinations and intermittent excretion of organisms, alternative diagnostic methods have been investigated.[3,5,6,10,11]
One important alternative has been the development of an antigen capture enzyme linked immunosorbent assay (ELISA) for use with stools. These tests have shown comparable sensitivity to experienced microscopic examinations, are fairly simple to perform and do not require the observation of intact organisms.[5,6,7,12]

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 – MT Plate**
   Microwells containing anti-*Giardia* antibodies
   96 test wells in a test strip holder.
2. **Negative control – Control -**
   1 vial, containing 2 ml of a *Giardia* negative formalinized stool supernatant.
3. **Positive control – Control +**
   1 vial, containing 2 ml of a diluted *Giardia* positive formalinized stool supernatant.
4. **Washing Buffer ⊥ BUF WASH 20x**
   1 bottle, containing 50 ml of (20x) concentrated buffer with detergent and Thimerosal.
5. **Enzyme conjugate – ENZ CONJ**
   1 bottle, containing 11 ml of peroxidase labeled anti-*Giardia* antibodies with Thimerosal.
6. **Specimen diluent – SPEC DIL**
   1 bottle, containing 60 ml of a buffered solution with detergent and Thimerosal.
7. **Chromogen/ Substrate – CHROMO SUB**
   1 bottle, containing 11 ml chromogen/substrate solution.
8. **Stop solution – STOP SOLN**
   1 vial, containing 11 ml (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) 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.

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. The Faecal Preparation Tube K6998SAS, Clindia Benelux 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 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 100µl of positive control to well # 2.*
3. Add 50µl of dilution buffer to each sample well. DO NOT add dilution buffer to control wells.
4. Add 50µl of sample to each well with dilution buffer.
5. Incubate for 60 minutes at room temperature (15-25º C), then wash**. After last wash slap the wells out on a clean absorbent towel to remove left over wash buffer.
6. Add 100µl of Enzyme Conjugate to each well.
7. Incubate for 30 minutes at room temperature (15-25º C), then wash**. After last wash slap the wells out on a clean absorbent towel to remove left over wash buffer.
8. Add 100µl of Chromogen to each well.
9. Incubate 10 minutes at room temperature (15-25º C). For automatic ELISA devices incubate 8 minutes at roomtemperature.
10. Add 100µl 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. 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
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.

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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