Giardia lamblia ELISA Kit

Enzyme Immunoassay for the determination of Giardia lamblia in human fecal specimens

Catalog number: ARG80554

For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

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. 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.

The mode of transmission of Giardia is through fecal-oral ingestion of cysts. Epidemics of giardiasis have been documented in day care centers and by drinking contaminated water. Day care centers may be directly or indirectly responsible for 45% of diagnosed Giardia infections in the United States. One study found 54% of the children at a day care center were infected.

Another important source of Giardia infection is among homosexual men. Prevalence rates of 5 to 19% for this population have been reported. 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.

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.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for Giardia lamblia has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any Giardia lamblia present is bound by the immobilized antibody. After washing away any unbound substances, a Horseradish Peroxidase (HRP) conjugated anti- Giardia lamblia Ab is added to each microplate well and incubated. After washing away any unbound antibody-enzyme reagent, a substrate solution (TMB) is added to the wells and color develops in proportion to the amount of Giardia lamblia bound in the initial step. The color development is stopped by the addition of acid and the intensity of the color is measured at a wavelength of 450 nm ±2 nm. The concentration of Giardia lamblia in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Antibody-coated microplate	1 plate	4 °C
Control Negative	2 ml (Ready to use)	4 °C
Control Positive	2 ml (Ready to use)	4 °C
HRP conjugated Antibody	11 ml (Ready to use)	4 °C (Protect from light)
Sample Diluent	60 ml (Ready to use)	4 °C
20X Wash buffer	50 ml	4 °C
TMB substrate	11 ml (Ready to use)	4 °C (Protect from light)
STOP solution	11 ml (Ready to use)	4 °C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Deionized or distilled water
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4 °C at all times.
- Briefly spin down the antibody conjugate concentrate and HRP-Streptavidin concentrate before use.

- If crystals are observed in the 20X Wash buffer, warm to RT (not more than 50 °C) until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.
- Samples contain azide cannot be assayed.

SAMPLE COLLECTION & STORAGE INFORMATION

Collection - 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.

Preparation –

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.

REAGENT PREPARATION

- 1X Wash buffer: Dilute 20X Wash buffer into distilled water to yield 1X Wash buffer.
- Samples: Samples have to be diluted 1:4 with Sample Diluent as information above.

  Note: the controls are ready-to-use and need not further dilution.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) before use. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add 100 µl of controls into control wells.
3. Add 50 µl of samples into sample wells.
4. Add 50 µl of Sample Diluent into sample wells. Incubate for 60 minutes at RT.
5. Aspirate each well and wash, repeating the process 2 times for a total 3 washes. Wash by filling each well with 1× Wash Buffer (350 µl) using a

squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.

6. Add 100 µl of HRP-antibody conjugated into each well. Cover wells and incubate for 30 minutes at RT.
7. Aspirate each well and wash as step 4.
8. Add 100 µl of TMB mixture to each well. Incubate for 10 minutes at room temperature in dark.
9. Add 100 µl of Stop Solution to each well.
10. Read the OD with a microplate reader at 450 nm immediately.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.

2. Using linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different 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.

ELASA Reader:

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.

QUALITY ASSURANCE

Quality control

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.

Intra-assay and Inter-assay precision

The CV value of intra-assay precision was 3.67% and inter-assay precision was 4.08%.