Code: HP007 giardiasis Ag
96 test

distribuito da:

GIARDIASIS Ag

Giardiasis Ag

The Cypress Diagnostics Giardia kit is an enzyme linked immunosorbent assay (ELISA) intended for the detection of Giardia antigen in fecal specimen.

General Information

Giardia lamblia is a flagellated enteric protozoan which infects mostly the small intestine after ingestion of Giardia cysts. Its distribution throughout the world makes it an important contributor to chronic debilitating diarrhea and to diarrhea in travelers. The acquisition of the parasite requires oral ingestion of Giardia cysts via fecal contaminated water or food. In the United States, it is the most prevalent infectious agent in waterborne outbreaks of diarrhea. In the developing world, Giardiasis is one of the first enteric pathogens infecting children less than 10 years of age with prevalence rates of 15-20 percent. Acquisition of Lambliasis occurs mainly in groups with poor fecal-oral hygiene via person-to-person transmission. Such ways of infection occur by children in day care centers, sexually active male homosexuals (up to 19%) and people in custodial institutions. Many infected young children are symptomatic and spread the disease within their homes and communities. The infection with cysts of the protozoan may be asymptomatic with older children, but they do harbor the cysts, excrete them intermittently and remain infectious to other people.

The Giardiasis is characterized as an acute or chronic diarrhea. The incubation period is 3 to 42 days. Clinical manifestations of symptomatic acute infection are sudden onset of watery diarrhea, abdominal cramps and flatulence. The patient expresses feelings of malaise, nausea and anorexia, less frequently vomiting and fever occur; blood, pus and musus are usually absent.

The diagnosis of Lambliasis in the past was done by stool examination for trophozoites or cysts by microscopy and means of staining. These methods require experienced lab personnel. In addition the investigation must be carried out over a time period since an intermittent excretion of the parasite can occur.

An equivalent method is the new ELISA test for the examination of Giardia lamblia antigen in stool specimen. He shows the same sensitivity as microscopy, needs no experienced personnel for microbiology, is easy and fast and needs no intact organisms (trophozoites or cysts) in stool specimens.

Principle of the test

On the surface of the microtiter wells, a monoclonal antibody against cell wall proteins (CWP) of Giardia lamblia cysts and trophozoites is bound.

Diluted stool samples and controls are pipetted into the wells.

A second monoclonal antibody cojugated to horseradish peroxidase is added and then incubated at room temperature.

The simulaneous incubation results in the Giardia lamblia antigen being sandwiched between the solid phase and enzyme-linked antibodies. Unbound POD conjugate is removed by washing.

Substrate (urea peroxide) and Chromogen (TMB) are added to the wells and incubated at room temperature.

The enzyme bound in the wells converts the colorless Substrate/Chromogen to a blue color.

Addition of Stop Solution converts the color from blue to yellow.

The absorption is measured at 450 nm wavelength (optional reference wavelength $\geq$ 600 nm).

The color intensity is directly proportional to the amount of antigen present in the sample.

Reagents provided

The reagents in one package are sufficient for 96 determinations.

Each test kit contains:

1 x 12 Microtiterstrips with 8 wells each (divisible) in a frame ; coated with monoclonal antibody (mouse) against Giardia lamblia; in a resealable foil bag

Vial 1 Universal Stool Diluent (100 ml); buffered NaCl solution for sample dilution; ready to use

Vial 2 Washing Buffer (100 ml; 10x conc., brown lid) pH 7.2, contains 0.1 % Thimerosal

Vial 3 Positive Control (1.2 ml); .Giarida lamblia antigen from human stool, heat inactivated ready to use.

Vial 4 Enzyme Conjugate (12 ml); HRP- conjugated mAb(mouse) against Giardia lamblia; dyed green, ready to use, contains 0.01 % Thimerosal

Vial A Substrate (6 ml); urea peroxide, ready to use

Vial B Chromogen (6 ml); tetramethylbenzidine (TMB), ready to use

Vial C Stop Solution (6 ml); 1 M sulfuric acid

Material needed but not provided

Distilled or deionized water

Test tubes

Transfer-Pipets

Vortex mixer

Micropipet for volumes of 100 µl and 1 ml

Microplate washer or multichannel pipet (250 µl)

Microplate reader (450 nm, optional reference wavelength ³ 600 nm)

Absorbent paper

Precautions

The Positive Control contains inactivated antigen of Giardia lamblia. However, the Positive Control as well as the Negative Control and the patient samples should be considered potentially contagious and be treated with the necessary safety precautions.

The Enzyme Conjugate and the Washing Buffer contain Thimerosal. Contact with skin or mucous membranes must be avoided.

Urea peroxide can cause cauterization. Handle with care !

The Stop Solution contains 1 M sulfuric acid. Avoid skin contact!

All reagents and materials coming in contact with potential infectious specimens must be treated with disinfectants or autoclaved at 121° C for at least one hour.

Except of the Universal Stool Diluent an exchange of individual reagents between kits of different lot numbers is not possible.

Storage

All reagents have to be stored at 2 - 8°C and can be used up to the expiry date printed on the labels. Microbial contamination has to be avoided. A quality warranty cannot be given beyond the kit expiration date.The diluted Washing Buffer has a shelf life of 4 weeks if stored at 2 - 8°C.

Allow reagents and Microwell Strips to get room temperature before use. To avoid moisture within the strips, do not take the strips out of the foil before having reached room temperature.

The foil bag should be opened with a pair of scissors without detaching the fastener. Return any unused strips to the foil bag, reseal and store them directly at 2-8°C.

The colorless Chromogen must be protected from exposure to direct light to avoid deterioration or coloration by autoxidation. If the Chromogen turns blue, the reagent should be discarded.

Indication of instability or deterioration.

The following criteria may indicate a reagent deterioration:

A turbidity or a blue coloration of the Chromogen prior to its use

An absorbance value of the Negative Control higher than 0.2

An absorbance value of the Positive Control lower than 0.8

Specimen collection and storage

Stool specimen can be used fresh or frozen. Fresh samples that have not been preserved should be stored at 4°C and should be tested within 24 h. Storage at 4°C of a specimen diluted in Stool Diluent can be prolonged for another 5 days at 2-8°C.

Samples which cannot be tested within this time period should be stored at -20°C until they are required. Deep freezing does not pose a negative influence on the test results.

Repeated thawing and freezing must be avoided.

Samples from MIF enrichment medium are not appropriate for ELISA processing as they may conduct to false positive results.

Test Procedure

-Preliminary comments

Bring all reagents and the Microwell Strips to room temperature before use. Mix the reagents well before use. Reproducibility in any EIA depends on exact pipetting, the observance of incubation times and temperature and the consistency of wash sequences.

During the washing steps, take care that all wells are filled with buffer and that the liquid is completely removed from the wells. Do not allow microwells to dry between steps.

Avoid direct sunlight during all incubations. Covering the microtiter plate is recommended.
Except the Washing Buffer, all reagents are ready to use.

-Preparing of the Washing Buffer

1 part of the concentrated Washing Buffer is diluted with 9 parts of distilled water.

Crystals in the buffer concentrate can be dissolved in a waterbath at 37°C. The diluted Washing Buffer has a shelf life of 4 weeks if stored at 2-8°C.

-Preparation of the samples

Using the Universal Stool Diluent (Vial 1) a 1:10 (v/v) dilution of a stool sample is made as followed:

Draw about 100 µl of liquid stool into a Pasteur pipet and suspend in 1 ml of the Universal Stool Diluent.

If the stool is solid, take an equivalent amount (volume of a pea) with a blade.

Homogenize sample by aspiration and ejection with a Transfer-Pipet or by mixing very thoroughly on a vortex-mixer. After allowing a short time to settle (max 10 minutes) stool suspension can be used directly in the test. If a longer time of settling has passed, the sample should be resuspended before use.

Remark: Using the Universal Stool Diluent the stool suspension can be applied to other Cypress Diagnostics EIAs for antigen detection in stool. For application in more than three assays a bigger volume of stool suspension should be prepared, for example 2 ml Universal Stool Diluent + 0.25 ml stool.

-First incubation

After a sufficient number of cavities has been placed into the frame, 100 µl of the Positive Control, the Universal Stool Diluent (Negative Control/ Vial 1)) and the diluted samples are pipetted into separate wells. Alternatively, man can pipet 2 drops with a Transfer-Pipet.

2 drops of Enzyme Conjugate (Vial4) are added to each well. Mix by gently swirling on tabletop and incubate at room temperature for 60 minutes.

-Washing

Decant or aspirate all wells into a waste container with a desinfectant. Ensure complete removal of the liquid from the wells by tapping the inverted plate onto absorbent paper. Fill 250 µl of prepared washing buffer in all wells. Repeat the wash cycle 5 times. Be sure to remove residual washing solution by firmly tapping the inverted microwells on absorbent paper after final washing.

If a microplate washer is used, stool suspension should be discarded manually. During the washing, be sure that the liquid is completely sucked off. After final washing step the inverted microwells should be firmly tapped on absorbent paper.

-Second incubation

Add 1 drop of Substrate (Vial A) and 1 drop of Chromogen (Vial B) into each well. Incubate the plate for 15 minutes at room temperature in the dark. Following the incubation, the reactions is stopped by adding 1 drop Stop Solution (Vial C) to each well. After carefully mixing (soft tapping on the edge of the plate) the absorbance is measured at 450 nm (optional reference wavelength $\geq$ 600 nm) against an air blank.

Remark: Highly positive patient samples can cause dark precipitates of the Chromogen.

Analysis

-Quality Control

For the quality control, the Positive and Negative Control must be included in each assay. The assay run is correct if the OD for the Negative Control is below 0.2 and the OD for the Positive Control is above 0.8.

-Calculation of the threshold (cut off)

The cut-off is determined by addition of 0.15 absorbance units to the measured absorption of the Negative Control

Cut-off = absorbance value of the Negative Control + 0.15

-Interpretation

Samples are considered positive if the absorbance value is higher than 10 % over the determined cut-off.

Samples, that have an absorbance value in the area of 10 % above or below the threshold should not be considered as clearly positive or clearly negative. They should be classified as indeterminate. It is recommended to test these samples again. As the repeated test with a fresh sample is indeterminate again the sample has to be considered negative.

Samples are considered negative if the absorbance value is lower than 10% under the determined cut-off.

-Remarks about the test procedure and interpretation

The Cypress Diagnostics Giardia assay detects Giardia lamblia antigen in stool specimens. A relation between the absorbance value and the clinical relevance is not given. Assay results should always be interpreted in connection to the clinical diagnosis.

A positive result does not exclude the presence of other pathogens.

A negative result does generally not exclude a Giardia infection. It can due to an intermittent excretion of the parasite. If a reasonable suspicion of an infection exist, a further stool specimen should be investigated.

An indeterminate result can be caused through an unequal dissemination of the parasite within the sample. In this case a second suspension from the same stool sample should be investigated or a further sample should be requested.

References

1. Black R. et al., Pediatrics, 60 (4) , 486 - 491 (1977).

2.Craun G.:Am.J Pub.Health 69 (No.8),817-819 (1979)

3. Nask, T.E. et al., J.Infect.Dis.156 (No.6), 974-984 (1987)

4.Smith,H.V. et al.,Microbiol.Eur. 3 (No1),22-29(1995)

5.Thompson,R.C.A.,Reynoldson,J.A., Adv.Parasitol.32,71-160 (1993)

6.Xiao, L., Parasitology today 10 (No.11),436-438 (1994)

Giardiasis Ag ELISA Ref. HP007

QUICK REFERENCE TEST PROCEDURE

1.Bring all reagents to room temperature

2. Dilute the Washing Buffer

3. Prepare the stool suspension

4. Pipet 100 µl (2 drops) of the suspension, the Positive and Negative Control into the microwells

5. Add 2 drops of Enzyme Conjugate; 60 minutes incubation at room temperature

6. Discard the incubate and wash 5 times with 250 µl of Washing Buffer

7. Add 1 drop each of Substrate and Chromogen; 15 minutes incubation at room temperature in the dark.

8. After addition of 1 drop Stop Solution spectrophotometric determination with a 450 nm filter.