E. histolytica/dispar Antigen Detection ELISA  
Cat. No. 8307-3

INTENDED USE

This ELISA is an *in vitro* immunoassay for the qualitative determination of *E. histolytica* antigen in feces. It is a double antibody (sandwich) ELISA using an anti-*E. histolytica* antibody to capture the antigen from the stool supernatant. A second anti-*E. histolytica* antibody is then added which sandwiches the captured antigen. This reaction is visualized by the addition of an anti-second antibody conjugated to peroxidase and the chromogen tetramethylbenzidine (TMB). The resulting blue color development indicates the presence of *E. histolytica* antigens being bound by the anti-*E. histolytica* antibodies.

SUMMARY AND EXPLANATION

*E. histolytica* is the protozoan parasite responsible for the disease amebiasis. Symptoms of acute amebiasis include diarrhea and colitis. The disease may manifest itself as an acute, chronic or as an asymptomatic infection. In addition, a percentage of the intestinal amebic infections will become extra-intestinal and cause abscesses in various organs. If extra-intestinal amebiasis is suspected, a serology test (such as IVD's *E. histolytica* Serology ELISA) should be used for diagnosis. By the time abscesses are occurring, the patient's stools are normally clear of amoebas.

The mode of transmission of *E. histolytica* is typically through fecal-oral ingestion of cysts, often by drinking contaminated water. Epidemics of amebiasis have been documented in developed nations but the parasite is quite common in under-developed countries. Travelers returning from under-developed countries account for the majority of cases in developed countries.

Diagnosis of intestinal amebiasis 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 PROCEDURE

During the first incubation, *E. histolytica* antigens present in the stool supernatant are captured by antibodies attached to the wells. The second incubation adds an additional anti-*E. histolytica* antibody that "sandwiches" the antigen. The next incubation adds an anti-second antibody conjugated to peroxidase. After washings to remove unbound enzyme, a chromogen is added which develops a blue color in the presence of the enzyme complex and peroxide. The stop solution ends the reaction and turns the blue color to yellow.
**WARNINGS/PRECAUTIONS**

- Do not add azides to the samples or any of the reagents.
- Do not use solutions if they precipitate or become cloudy.
- Thaw sample if needed. Add sufficient diluted wash buffer to make approximately a 1:4 dilution (1 gram or a pea size of fecal sample to 3 ml of diluted wash buffer) and mix well. Allow heavy precipitates to settle.

**STORAGE CONDITIONS**

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

**SPECIMEN COLLECTION AND PREPARATION**

- **Collection of Stool (Feces)**
  1. No modification of collection techniques used for standard microscopic O&P examinations is needed. Stool samples may be used as unprocessed or frozen, or in preservation media of 10% formalin.
  2. Unprocessed samples should be kept at 2 - 8ºC and tested within 24 hours of collection. Samples that cannot be tested within this time should be frozen at –15º to –20ºC or lower until used. Freezing does not adversely affect the test.
  3. Formalized and SAF samples can not be used in this assay.
  4. All dilutions of unprocessed stools must be made with diluted wash buffer.
  5. Wash Buffer Preparation – Remove cap and add contents of one bottle of Wash Concentrate to a squeeze bottle containing 475 ml of DI water. Swirl to mix. Squeeze bottle should have a narrow tip to optimize washings.

- **Preparation of Fresh/Frozen Stools**
  Thaw sample if needed. Add sufficient diluted wash buffer to make approximately a 1:4 dilution (1 gram or a pea size of fecal sample to 3 ml of diluted wash buffer) and mix well. Allow heavy precipitates to settle.

**PROCEDURE**

**Reagent Preparation:**

1. Bring a bottle of the 20X Wash Solution to 500mL with distilled water. Mix well.

**Assay Procedure:**

1. Break off the required number of wells needed (number of samples plus 2 for controls) and place in holder.
2. Add 2 drops (approximately 100 ul) of negative control to well #1 and 2 drops of positive control to well #2.
3. Add 100uL of the stool supernatant to the appropriate test well.
4. Incubate for 30 minutes at room temperature (15-25º C), then wash.*
5. Add 2 drops of Reagent 1 (Blue soln) to each well.
6. Incubate for 5 minutes at room temperature, then wash.
7. Add 2 drops of Reagent 2 (Red soln) to each well.
8. Incubate for 5 minutes, then wash.
9. Add 2 drops of Chromogen Solution to each well.
10. Incubate at room temperature for 5 minutes. DO NOT WASH.
11. Add 2 drops of Stop Solution to each well. Mix by gently tapping the sides of the plate.
12. Read results visually or at 450/620-650 nm. Zero reader on air.

* Washings consist of vigorously filling each well to overflowing and decanting contents three separate times.

Controls must be included each time the kit is run.

**MATERIALS REQUIRED BUT NOT PROVIDED**

- Transfer Pipettes
- Squeeze bottle for washing strips (narrow tip is recommended)
- Graduated Cylinder
- Reagent grade (DI) water
- Suggested Equipment
  - ELISA plate reader with 450 and 620-650 nm filters
  - Proper Temperature
- All incubations are at room temperature (15 to 25º C)

**REAGENTS**

- **MICROWELL PLATE** 1plate
  - 12x8/8 x 12-well strips per plate fixed on white strip holder.
  - The plate is sealed in a pouch with desiccant.
  - Each well contains anti-E. histolytica polyclonal antibodies. The microwell strips can be broken to be used separately. Place unused wells or strips in the plastic sealable storage bag together with the desiccant and return to 2-8ºC.
- **REAGENT 1** 1bottle
  - 11ml per bottle
  - Monoclonal anti-E. histolytica antibodies with blue dye and Thimerosal
  - Ready to use as supplied.
  - Once open, stable for one month at 2-8ºC.
- **REAGENT 2 (HRP)** 1bottle
  - 11mL per bottle
  - Anti-mouse antibodies conjugated to horseradish peroxidase with red dye and Thimerosal.
  - Ready to use as supplied.
  - Once open, stable for one month at 2-8ºC.
- **NEGATIVE CONTROL** 1vial
  - 1 ml per vial.
  - Stool buffer.
  - Ready to use as supplied.
  - Once open, stable for one month at 2-8ºC.
- **POSITIVE CONTROL** 1vial
  - 1 ml per vial.
  - Diluted E. histolytica antigen in buffer
  - Ready to use as supplied.
  - Once open, stable for one month at 2-8ºC.
- **WASH BUFFER (20X)** 2 bottles
  - 25ml per bottle.
  - 20X concentration buffer with surfactant and Thimerosal
  - DILUTE BEFORE USE - The concentrate must be diluted with distilled/deionized water before use.
  - Once diluted, stable for one week at room temperature or for two weeks at 2-8ºC.
- **CHROMOGEN SOLUTION** 1bottle
  - 11ml per bottle.
  - TMB (tetramethylbenzidine) and peroxide.
  - Ready to use as supplied.
  - Once open, stable for one month at 2-8ºC.
- **STOP SOLUTION** 1bottle
  - 11ml per bottle.
  - 1M phosphoric acid in water.
- **PLASTIC SEALABLE BAG** 1unit
  - For enclosing the strips not in use.
- **PACKAGE INSERTS** 1copy

**E. histolytica/dispar ELISA**

**Version: Original (02-22-06)**
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
Zero reader on air. Read all wells at 450/620-650 nm.
Reactive: Absorbance reading of 0.15 OD units and above indicates the sample contains E. histolytica/dispar antigen.
Non-reactive: Absorbance reading less than 0.15 OD units indicates the sample does not contain detectable levels of E. histolytica/dispar antigen.

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 0.15 OD units. Should the value fall below this limit, the kit should not be used.

EXPECTED VALUES
Normal healthy individuals should be free of E. histolytica/dispar and should test negative. A positive reaction indicates that the patient is shedding detectable amounts of E. histolytica/dispar or E. dispar antigen.

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

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 E. histolytica/dispar.

PERFORMANCE CHARACTERISTICS
Study #1 – vs. Microscopy
N = 46

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Sensitivity – 7/8 = 88%
Specificity – 38/38 = 100%

VALIDITY
Please do not use this kit beyond the expiration indicated on the kit box and reagent labels.