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 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.

REAGENTS
• Test strips: microwells containing anti-E. histolytica polyclonal antibodies - 96 test wells.
• Test strip holder: One (1).
• Ab: One (1) bottle containing 11 ml of a monoclonal anti-E. histolytica antibody with blue dye and Thimerosal.
• Conjugate: One (1) bottle containing 11 ml of anti-mouse-peroxidase with red dye and Thimerosal.
• Positive control: One (1) vial containing 1 ml of diluted E. histolytica antigen in buffer.
- **Negative control:** One (1) vial containing 1 ml of stool buffer.
- **TMB Substrate:** One (1) bottle containing 11 ml of the chromogen tetramethylbenzidine (TMB) and peroxide.
- **Wash Concentrate 20X:** Two (2) bottles containing 25 ml of concentrated buffer and surfactant with Thimerosal.
- **Stop solution:** One (1) bottle containing 11 ml of 1M phosphoric acid.

**PRECAUTIONS**

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

**STORAGE CONDITIONS**

*Reagents, strips and bottled components:*

- Store between 2 - 8°C.
- Squeeze bottle containing diluted wash buffer may be stored at room temperature.

**PREPARATION**

**Wash Buffer**

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.

**Specimen Collection And Preparation**

1. **Collection of Stool (Feces)**

   No modification of collection techniques used for standard microscopic O&P examinations is needed.
   Stool samples may be used as unpreserved or frozen.
   Preserved stools cannot be used in this assay.
   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 and SAF preserved samples cannot be used in this assay.
   All dilutions of stools must be made with diluted wash buffer.

2. **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.
MATERIALS REQUIRED BUT NOT PROVIDED
1. Transfer Pipettes
2. Squeeze bottle for washing strips (narrow tip is recommended)
3. Graduated Cylinder
4. Reagent grade (DI) water
5. ELISA plate reader with 450 and 620-650 nm filters

TEST PROCEDURE
All incubations are at room temperature (15 to 25°C)
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 2 drops of the stool supernatant to each test well.
4. Incubate for 30 minutes at room temperature (15-25°C), then wash.*
5. Add 2 drops of Reagent 1 (blue solution) to each well.
6. Incubate for 5 minutes, then wash.
7. Add 2 drops of Reagent 2 (red solution) to each well.
8. Incubate for 5 minutes, then wash.
9. Add 2 drops of Chromogen to each well.
10. Incubate 5 minutes.
11. Add 2 drops of Stop Solution to each well. Mix wells by gently tapping the side of the strip holder with index finger.
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.

RESULTS
a. 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.

b. 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 antigen.
   Non-reactive: Absorbance reading less than 0.15 OD units indicates the sample does not contain detectable levels of E. histolytica antigen.
LIMITATION OF PROCEDURE
1. Test results should be used as an aid in diagnosis and should not be interpreted as diagnostic by themselves.
2. DO NOT concentrate stool samples. Assay will not give accurate results on a concentrated sample.
3. 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.

PERFORMANCE DATA

Study #1 – vs. Microscopy
N = 46

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

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

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

BIBLIOGRAPHY