DIAGNOSTIC AUTOMATION, INC.

21250 Califa Street, Suite 102 and 116, Woodland Hills, CA 91367
Tel: (818) 591-3030 Fax: (818) 591-8383
onestep@rapidtest.com
technicalsupport@rapidtest.com
www.rapidtest.com

IVD | See external label | 2°C–8°C | 96 tests | REF 8307-3

E. histolytica/dispar

REF **8307-3**

Test	**E. histolytica/dispar Antigen ELISA**
Method	**Enzyme Linked Immunosorbent Assay**
Principle	**Double Antibody Sandwich Assay**
Detection Range	**Qualitative: Positive/Negative**
Sample Size	**100 µl**
Specimen	**Unpreserved or Frozen Stool**
Specificity	**100%**
Sensitivity	**88%**
Total Assay Time	**45 minutes**
Shelf Life	**12 Months from the manufacturing date**

** Laboratory results can never be the only base of a medical report. The patient history and further tests have to be taken into account*

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 DAI'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.

Material Provides:

Item	Description	Symbol
Test Strips	Microwells containing anti-*E. histolytica* antibodies: 96 test wells in a test strip holder.	MT PLATE
Reagent 1	One (1) bottle containing 11 ml of *anti-E. histolytica* antibodies with blue dye and Thimerosal.	Ab
Reagent 2	One (1) bottle containing 11 ml of antibodies conjugated to horseradish peroxidase with red dye and Thimerosal.	CONJ
Positive Control	One (1) vial containing 2 ml of diluted *E. histolytica* antigen in buffer.	CONTROL +
Negative Control	One (1) vial containing 2 ml of buffer.	CONTROL −
Chromogen	One (1) bottle containing 11 ml of the chromogen tetramethylbenzidine (TMB) and peroxide.	SUBS TMB
Wash Concentrate (20X)	Two (2) bottles containing 25 ml of concentrated buffer and surfactant with Thimerosal.	WASH BUF
Stop Solution	One (1) bottle containing 11 ml of 5% phosphoric acid solution.	SOLN

Materials Required But Not Provided

Transfer Pipettes
Squeeze bottle for washing strips (narrow tip is recommended)
Graduated Cylinder
Reagent grade (DI) water
Sample dilution tubes
ELISA plate reader with 450 and 620-650 nm filters

Warnings/Precautions

- **Do not deviate from the specified procedures when performing this assay**. All specimen dilutions, incubation times/temperatures and washings have been optimized for the best performance characteristics. Deviations from the specified procedures may affect the sensitivity and specificity of the assay.
- For In Vitro Diagnostic Use Only.
- Do not interchange reagents between kits with different lot numbers.
- Do not use reagents that are beyond their expiration dates. Expiration dates are on each reagent label. Use of reagents beyond their expiration dates may affect results.
- Unused microwells should be stored in the desiccated pouch to protect them from moisture.

- 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, which may be irritating to skin, eyes and mucous membranes. In case of contact, flush eyes or rinse skin with copious amounts of water.
- Treat all reagents and samples as potentially infectious materials. Use care to prevent aerosols and decontaminate any spills of samples.
- 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.
- 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.

For research use only.
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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 ºC.
Squeeze bottle containing diluted wash buffer may be stored at room temperature.

Preparation

- Before use, bring all reagents and samples to room temperature (15-25 °C) and mix.
- (20X) Wash Concentrate may precipitate during refrigerated storage, but will go back into solution when brought to room temperature (15-25°C) and mixed. ***Ensure that (20X) wash concentrate is completely in solution before diluting to working concentration***. To dilute (20X) wash concentrate to working dilution, 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

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 ºC and tested within 24 hours of collection. Samples that cannot be tested within this time should be frozen at -20 ºC or lower until used. Freezing does not adversely affect the test however, avoid repeated freeze/thaw cycles. Formalized and SAF preserved samples cannot be used in this assay. All dilutions of stools must be made with diluted wash buffer.
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.

PROPER TEMPERATURE

All incubations are at room temperature (15 to 25 ºC)

TEST PROCEDURE

Notes:

- Ensure all samples and reagents are at room temperature (15-25 °C) before use. Frozen samples must be thawed completely before use.
- When running the assay, try to avoid the formation of bubbles in the wells. Bubbles may affect overall performance and reading of end results. Slapping the wells out on a clean absorbent towel after each wash step should help to minimize bubbles in the wells.
- Controls must be included each time the kit is run. Controls are provided prediluted. DO NOT dilute further.

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.
3. Add **100 µl** of positive control to well # 2.
4. Add **100 µl** of the stool supernatant to each test well.
5. Incubate for **30 minutes** at room temperature (15-25 ºC), then wash.* After last wash slap the wells out on a clean absorbent towel to remove excess wash buffer.
6. Add **2 drops** of Reagent 1 (blue solution) to each well.
7. Incubate for **5 minutes**, then wash*. After last wash slap the wells out on a clean absorbent towel to remove excess wash buffer.
8. Add **2 drops** of Reagent 2 (red solution) to each well.
9. Incubate for **5 minutes**, then wash*. After last wash slap the wells out on a clean absorbent towel to remove excess wash buffer.
10. Add **2 drops** of Chromogen to each well.
11. Incubate **5 minutes**.
12. Add **2 drops** of Stop Solution to each well. Mix wells by gently tapping the side of the strip holder with index finger.
13. Read results visually or using an ELISA plate reader (see instructions below).

** Washings consist of vigorously filling each well to overflowing and decanting contents three separate times. When possible, avoid formation of bubbles in the wells as this may affect the end results.*

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.

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

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*.

Performance Data

Study #1 – vs. Microscopy
N = 46

		Microscopy	
		+	-
DAI	+	7	0
	-	1	38

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 positive and negative control must be included each time the kit is run. The use of a positive and negative control allows easy validation of kit stability.

- Negative control should appear colorless to faintly yellow when read visually and should read less than 0.15 OD when read at a dual wavelength of 450/620-650 nm.
- Positive control should be a clearly visible yellow color and read greater than 0.5 OD when read at a dual wavelength of 450/620-650 nm.

TROUBLESHOOTING

Problem*:* Negative control has excessive color after development.
Reason*:* Inadequate washings

Correction: Wash more vigorously. Remove excessive liquid from the wells by tapping against an Absorbent towel. Do not allow test wells to dry out

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