



AccuDiag™ E. histolytica/dispar Antigen ELISA Kit

REF 8307-3

IVD See External Label 8°C 96 Tests

E. histolytica/dispar Antigen ELISA	
Principle	Sandwich ELISA
Detection	Qualitative
Sample	100 µl
Incubation Time	45 minutes
Sensitivity	88%
Specificity	100%
Shelf Life	12 Months from the manufacturing date

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

PRODUCT FEATURES

- Very easy to use with little training
- Highly specific and consistent Assay
- Provides accurate results quickly
- Reading of results both visually and as absorbance data

INTENDED USE

This ELISA is an *in vitro* immunoassay for the qualitative determination of *E. histolytica* antigen in feces.

SIGNIFICANCE AND SUMMARY

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.

ASSAY PRINCIPLE

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.

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°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 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.

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.

REAGENTS

Materials provided with the kit

1. *E. histolytica/dispar* Stool Antigen Detection Microwell ELISA kit
2. Test Strips: Microwells containing anti-*E. histolytica* antibodies: 96 test wells in a test strip holder.
3. Reagent 1: One (1) bottle containing 11 ml of anti-*E. histolytica* antibodies with blue dye and Thimerosal.
4. Reagent 2: One (1) bottle containing 11 ml of antibodies conjugated to horseradish peroxidase with red dye and Thimerosal
5. Positive Control: One (1) vial containing 2 ml of diluted *E. histolytica* antigen in buffer
6. Negative Control: One (1) vial containing 2 ml of buffer.



7. Chromogen: One (1) bottle containing 11 ml of the chromogen tetramethylbenzidine (TMB) and peroxide.
8. Wash Concentrate (20X): Two (2) bottles containing 25 ml of concentrated buffer and surfactant with Thimerosal.
9. Stop Solution: One (1) bottle containing 11 ml of 5% phosphoric acid solution.

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. Sample dilution tubes

Suggested Equipment

ELISA plate reader capable of reading bichromatically at 450/620-650 nm

REAGENT 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.

ASSAY PROCEDURE

- All incubations are at room temperature (15 to 25°C)
 - 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°C), 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.

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.

RESULTS AND INTERPRETATIONS

Interpretation of Results - Visual

Positive: Any sample well that is obviously more yellow than the negative control well.

Negative: 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.

Positive: Absorbance reading of 0.15 OD units and above indicates the sample contains *E. histolytica* and/or *E. dispar* antigen.

Negative: Absorbance reading less than 0.15 OD units indicates the sample does not contain detectable levels of *E. histolytica* or *E. dispar* antigen.

QUALITY CONTROL

The negative control and positive controls must be included each time the assay is run. The use of positive and negative controls allows easy validation of kit stability readings at dual wavelength of 450/620-650.

Negative - 0.0 to 0.15 OD units

Positive - 0.5 OD units and above

EXPECTED RANGES OF VALUE

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.

PERFORMANCE CHARACTERISTICS

Study #1 – vs. Microscopy

N = 46

		Microscopy	
		+	-
Diagnostic Automation Inc.	+	7	0
	-	1	38

Sensitivity – 7/8 = 88%

Specificity – 38/38 = 100%



LIMITATIONS OF THE ASSAY

- 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*.

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.

STORAGE CONDITIONS

- Reagents, strips and bottled components: Store between 2 – 8°C.
- Squeeze bottle containing diluted wash buffer may be stored at room temperature (15 – 25°C).

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MANUFACTURER AND BRAND DETAILS

ISO 13485:2016

ISO 13485
Quality
Management for
Medical Devices
CERTIFIED

Diagnostic Automation/Cortez Diagnostics, Inc.
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Brand Name	AccuDiag™
REF 8307-3	AccuDiag™ - <i>E. histolytica/dispar</i> Antigen ELISA
EC REP	CEpartner4U, Esdoornlaan 13, 3951 DB Maarn, The Netherlands. www.cepartner4u.eu
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