E. histolytica
ELISA [Enzyme-Linked ImmunoSorbent Assay]

Catalog # 7078
Qualitative assay for the determination of
E. histolytica/dispar antigen in human feces

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BIOMERICA

I. INTENDED USE

The Biomerica *E. histolytica/dispar* ELISA is intended for the qualitative detection of *Entamoeba* specific antigen in fecal specimens. This assay is intended for *in vitro* diagnostic use only.

II. SUMMARY AND EXPLANATION

Entamoeba histolytica is an anaerobic parasitic protozoan, part of the genus *Entamoeba*. *E. histolytica* and *E. dispar* are virtually indistinguishable species which are thought to infect up to 500 million people worldwide.[1,2,3]. *E. histolytica*, the pathogenic species, infects approximately 50 million people worldwide.

Clinical symptoms in people infected with *E. histolytica* range from non-specific symptoms of gastrointestinal disease to dysentery, colitis, and amebiasis. Symptoms of amebiasis include cramp-like abdominal pains, nausea, and severe diarrhea – often bloody. Approximately 10% of cases of acute amebic dysentery result in extra-intestinal complications, such as hepatic abscesses, pulmonary abscesses, or even cerebral abscesses.[5] If these more serious complications are not treated, death is often the result, and deaths attributed to *E. histolytica* infection are estimated to be approximately 80,000 per year worldwide.[3]

E. histolytica exists either as a trophozoite (active) stage which exists only in the host in fresh, loose feces, or as cysts, which are very stable outside of the host in water, soils, and on moist foods. Thus, transmission occurs through the ingestion of contaminated water or foods. *E. histolytica* can also be transmitted through anal-oral sex. Research has shown that HIV-infected gay men are at greater risk of infection than the heterosexual population.

Diagnosis of amebiasis has been done through a variety of non-invasive techniques, the most common of which has been microscopic examination of fecal specimens. However, this requires an experienced technician, and very often, multiple stool specimens. Various studies have shown that correct diagnosis by microscopy is only achieved 50-76% of the time.[6,7] More sensitive and specific procedures, such as ELISA, have been developed which eliminate the subjective evaluation associated with microbiology.[8,9,10]

III. PRINCIPLE OF THE TEST

The Biomerica *E. histolytica* test is an enzyme immunoassay which detects the presence of *Entamoeba* specific antigen (ESA) in stool samples. Antibodies to ESA have been immobilized on breakaway microwells. Diluted patient specimens are added to the microwells along with an additional *E. histolytica* antibody that 'sandwiches' the antigen. After washing, an anti-second antibody conjugated to horseradish peroxidase is added. If ESA is present in the sample, the 'sandwich' complex will bind to the detecting antibody, which will remain in the microwell after washing to remove unbound enzyme. After washing, a substrate/chromogen is added which develops a blue color in the presence of the enzyme complex. The stop solution ends the reaction and turns the blue color to yellow.

IV. KIT COMPONENTS

Kit Components	Description	Symbol
Test Strips	96 microtiter wells containing anti-*E. histolytica* polyclonal antibodies in a test strip holder.	**PLA**
Antibody Reagent	One (1) bottle containing 11 ml of a monoclonal *anti-E. histolytica* antibody with blue dye and thimerosal.	**AB**
Enzyme Reagent	One (1) bottle containing 11 ml of anti-mouse-peroxidase with red dye and thimerosal.	**CONJ ENZ**
Positive Control	One (1) vial containing 2 ml of inactivated *E. histolytica* antigen in buffer.	**CTRL +**
Negative Control	One (1) vial containing 2 ml of a buffer.	**CTRL -**
Substrate Solution	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.	**BUF WASH 20X**
Stop Solution	One (1) bottle containing 11 ml of 5 % phosphoric acid.	**STOP**

MATERIAL AND EQUIPMENT REQUIRED BUT NOT PROVIDED

- Microplate reader (Optional).
- Microplate washer [if washer is unavailable, manual washing may be acceptable].
- Micropipette capable of delivering 100 µL.
- Transfer Pipettes
- Graduated Cylinder
- Wash Bottle.
- Absorbent Paper.
- Timer
- Reagent grade (DI) water.
- Waste container with disinfectant or biohazard bags.
- Sample Dilution Tubes.
- Applicator Stick (recommended) or swabs for sample preparation.
- Parafilm or cover for microwell plate

V. WARNINGS AND PRECAUTIONS FOR USERS

- **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. Deviations from the specified procedures may affect the sensitivity and specificity of the assay.
- All reagents are for *In Vitro* Diagnostic Use Only.
- Reagents from different kit lots should not be interchanged.
- Do not use reagents that are beyond their expiration dates.
- All reagents should be at room temperature before using.
- If using dropper bottles, hold them vertically to ensure proper drop size.
- Wear gloves when performing the test, and handle specimens and used microwells as if able to transmit infectious agents.
- Unused microwells should be stored in the resealable pouch with desiccant 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.
- 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 large amounts of water.
- Treat all reagents and samples as potentially infectious materials. Use care to prevent aerosols and decontaminate any sample spills.
- Stop solution is a 5% solution of phosphoric acid in water. In case of contact with skin or mucous membranes, flush with water immediately.

VI. SAMPLE COLLECTION AND STORAGE

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. Formalinized and SAF preserved samples cannot be used in this assay.

VII. REAGENT PREPARATION AND STORAGE

1. All reagents, with the exception of the Wash Concentrate are supplied ready-to-use. All reagents should be stored at 2-8 °C. The Wash Concentrate may precipitate during refrigerated storage but will dissolve upon warming.
2. Before use, bring all reagents and samples to room temperature (15-25°C) and mix.
3. Wash Concentrate: To prepare a 1X wash solution, add contents of one bottle of Wash Concentrate (25 ml) to 475 ml of distilled or deionized water and mix.

VIII. SAMPLE PREPARATION

Preparation of Fresh/Frozen Stools

1. Thaw sample if needed. Add sufficient amount of 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.
2. **IF USING SWABS**, add **1 ml** of diluted wash buffer to dilution tube. Coat the swab with a thin layer of specimen and mix into dilution buffer, expressing as much fluid as possible. Mix thoroughly before using.
3. **For watery unpreserved specimens**, mix contents then add **100 µl** of sample to **700 µl** of diluted wash buffer in dilution tubes. Mix thoroughly before using.

NOTES:

- All dilutions of stools must be made with diluted wash buffer
- Ensure all samples and reagents are at room temperature (15-25 °C) before use. Frozen samples must be thawed completely before use.
- If needed, prepared samples can be centrifuged at 2000-3000 g for 5-10 minutes. Ensure supernatant is clear before use.

IX. ASSAY PROCEDURE

NOTES:

- All incubations are at room temperature (15 to 25 °C)
- When running the assay, try to avoid the formation of bubbles in the wells. Slapping the wells out on a clean absorbent towel after each wash step should help to minimize bubbles in the wells.

1. Break off the required number of wells needed (number of samples plus 2 for controls) and place in holder. Return any unused wells to foil pouch with desiccant. Reseal pouch tightly to exclude moisture.
2. Using a micropipette, add **100 µl (or 2 drops)** of negative control to well # 1.
3. Using a micropipette, add **100 µl (or 2 drops)** of positive control to well # 2.
4. Add **100 µl** of sample to each well.

NOTE: Place the opening of the transfer pipette just inside the well to avoid splashing into adjacent wells.

5. Cover the plate with parafilm or an appropriate cover and incubate for **30 minutes** at room temperature (15-25 °C). Begin the timing after the addition of the last specimen.
6. Decant the contents of the assay wells.
7. Wash each well using the 1X wash solution in a squirt bottle with a fine-tipped nozzle, directing the wash solution to the bottom of the well with force. Fill the well, and then decant the wash solution out of the well. Repeat this step two more times for a total of three washes. *

NOTE: If using semi-automated or automated washing equipment, the specimens must be centrifuged (2000 to 3000g x 10 minutes) to remove any particulate matter prior to adding to the plate. Add 350 µL of 1X wash solution to each well. Wash a total of three times.

8. Add **100 µl (or 2 drops)** of Antibody Solution (blue) to each well.
9. Cover the microwell plate and incubate at room temperature for **15 minutes**.
10. Decant the contents of the wells and wash each well three times as in **Step 6-7***.
8. Add **100 µl (or 2 drops)** of Enzyme Conjugate (red) to each well.
11. Cover the microwell plate and incubate for **15 minutes** at room temperature.
12. Decant the contents of the wells and wash each well three times as in **Step 6-7***.
10. Add **100 µl (or 2 drops)** of Substrate Solution to each well.
11. Cover the plate and incubate for **15 minutes** at room temperature.
12. Add **100 µl (or 2 drops)** of Stop Solution to each well. Gently tap the wells to mix for approximately **15 seconds**. Read reaction within **5 minutes** after adding stop solution.
13. Read results visually or with a spectrophotometer having a dual wavelength of 450/620-650 nm. Zero reader on air.

Washings consist of vigorously filling each well to overflowing and decanting contents, banging the wells on a clean absorbent towel after each wash.

X. CALCULATION OF RESULTS

Interpretation of Results – Visual Method

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 - Spectrophotometric Method:

Zero spectrophotometer against air**. Read all wells at 450/620-650 nm.**

Positive: Absorbance reading of 0.15 O.D. units and above indicates the sample contains *Entamoeba* antigen.

Negative: Absorbance reading less than 0.15 O.D. units indicates the sample does not contain detectable levels of *E*ntamoeba antigen.

XI. QUALITY CONTROL

Positive and negative controls must be included each time the assay is run. The use of a positive and negative control provides an easy validation of kit stability.

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

XII. LIMITATION OF PROCEDURE

- This test does not distinguish *E. histolytica* from *E. dispar*.
- 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.
- Inadequate washings may lead to the negative control having excessive color development. Care should be taken to perform wash steps in the manner described.
- 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*.

XIII. PERFORMANCE CHARACTERISTICS

Clinical Evaluation

Study 1

A total of 83 stool specimens were tested on the Biomerica *E. histolytica* ELISA and a reference ELISA for comparison. The clinical profile for the Biomerica *E. histolytica* ELISA is shown below.

		Reference *E.histolytica* ELISA		
		POS	NEG	TOTAL
BIOMERICA *E. histolytica*	POS	26	0	26
	NEG	0	57	57
		26	57	83

Accuracy	100%
Sensitivity	100%
Specificity	100%

Study 2

In a second study 67 confirmed negative stool samples and 26 confirmed positive stool samples were tested on the Biomerica *E. histolytica* ELISA. All samples were confirmed by microscopy examination. The clinical profile for the Biomerica *E. histolytica* ELISA is shown below.

		Microscopy Examination		
		POS	NEG	TOTAL
Biomerica *E. histolytica* ELISA	POS	23	0	23
	NEG	3	67	70
		26	67	93

Accuracy	97%
Sensitivity	88%
Specificity	100%

Precision and Reproducibility

The precision (Intra-assay variation) of the Biomerica *E. histolytica* ELISA test was calculated from 24 replicate determinations on each of the two control samples

Intra Assay

Sample	Mean O.D	N	% CV
Control 1	1.1	24	4.2%
Control 2	1.6	24	4.8%

Inter Assay

The reproducibility (inter-assay variation) of the Biomerica *E. histolytica* ELISA test was calculated from data on two control samples obtained in 12 different assays, by three technicians on two different lots of reagent

Sample	Mean O.D	N	% CV
Control 1	0.9	12	9.3%
Control 2	1.3	12	9.9 %

Cross-Reactivity

The following bacterial strains, greater than 10^6 cell/mL, were spiked into a confirmed negative fecal specimen. No cross-reactivity to the below bacterial strains was observed in the Biomerica *E. histolytica* ELISA.

Bacterial Strain	ATCC #	OD_{450nm}-OD_{630nm}
Negative Sample - No Spike	N/A	0.001
Campylobacter coli 1114 - Spike	N/A	0.001
Campylobacter fetus - Spike	19438	0.003
Escherichia coli Serotype 055:k59 (B5) - Spike	12014	0.002
Escherichia coli Serotype 0111:K58 (B4) - Spike	33780	0.004
Escherichia col i Serotype 0111:NM - Spike	43887	0.004
Escherichia coli Serotype 0157.H7 - Spike	43890	0.002
Escherichia coli Serotype 0124.NM - Spike	43893	0.004
Campylobacter jejuni - Spike	29428	0.001
Salmonella typhimurium - Spike	SA972229	0.003
Shigella sonneii - Spike	25931	0.006

Interference

The following bacterial strains, greater than 10^6 cell/mL, were spiked into a confirmed positive fecal specimen. No interference to the below bacterial strains was observed in the Biomerica *E. histolytica* ELISA, as all positive samples remained positive.

Bacterial Strain	ATCC #	OD_{450nm}-OD_{630nm}
Positive Sample - No Spike	N/A	3.900
Campylobacter coli 1114 - Spike	N/A	3.730
Campylobacter fetus - Spike	19438	3.896
Escherichia coli Serotype 055:k59 (B5) - Spike	12014	3.861
Escherichia coli Serotype 0111:K58 (B4) - Spike	33780	3.881
Escherichia col i Serotype 0111:NM - Spike	43887	3.900
Escherichia coli Serotype 0157.H7 - Spike	43890	3.900
Escherichia coli Serotype 0124.NM - Spike	43893	3.805
Campylobacter jejuni - Spike	29428	3.900
Salmonella typhimurium - Spike	SA972229	3.669
Shigella sonneii - Spike	25931	3.799

XIV. REFERENCES

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XV. SYMBOLS

	Storage Temperature
	Lot Code
	Expiration
	Manufacturer
	Authorized Representative
	Caution, see instructions
	For in vitro diagnostic use
	Catalog No.

XVI. ORDERING INFORMATION

ORDERING: Send purchase order to:
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"Authorized Representative"
according to IVDD 98/79/ EC

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