# DRG® Entamoeba histolytica IgG (Amebiasis) (EIA-3474)

IVD

**Revised 23 May 2011 rm (Vers. 7.1)**

***Please use only the valid version of the package insert provided with the kit.***

## Intended Use

For the qualitative screening of serum IgG antibodies to *Entamoeba histolytica* using an Enzyme Linked Immunoabsorbant Assay (ELISA) technique.
**For In Vitro Diagnostic Use.**

## Summary

Amebiasis is the disease caused by the protozoan parasite *Entamoeba histolytica*. This organism is endemic throughout the world in developing countries, and can be found in immigrants and travelers from these areas. The disease usually manifests with intestinal symptoms. In a minority of cases, the organism will become extra-intestinal and lead to abscess formation in different organs. Of the organs that could be affected, the liver is the most common site. Typically, the organism can no longer be found in the feces once the disease goes extra-intestinal. Serological tests are useful in detecting infection by *E. histolytica* if the organism goes extra-intestinal and in excluding the organism from the diagnosis of other disorders (e.g. chronic liver diseases, ulcerative colitis, etc.). This serology test should not be used for detecting intestinal infections. An Ova & Parasite (O&P) test or an *E. histolytica* fecal antigen assay is the proper assay for intestinal infections.
Since antibodies may persist for years after clinical cure, a positive serological result may not necessarily indicate an active infection. A negative serological result however can be equally important in excluding suspected tissue invasion by *E. histolytica*.

## Principle of Procedure

The micro test wells are coated with *E. histolytica* antigen. During the first incubation with the diluted patients' sera, any antibodies which are reactive with the antigen will bind to the coated wells. After washing to remove the rest of the sample, the Enzyme Conjugate is added. If antibodies have been bound to the wells, the Enzyme Conjugate will then bind to these antibodies. After another series of washes, a chromogen (tetramethylbenzidine or TMB) is added. If the Enzyme Conjugate is present, the peroxidase will catalyze a reaction that consumes the peroxide and turns the chromogen from clear to blue. Addition of the Stop Solution ends the reaction and turns the blue color to a bright yellow color. The reaction may then be read visually or with an ELISA reader.

**Reagents**

| Item | Description | Symbol |
|---|---|---|
| Test Strips | Microwells containing E. histolytica strain NIH-200 antigens - 96 test wells in a test strip holder. | MT PLATE |
| Enzyme Conjugate | One (1) bottle containing 11 ml of Protein A conjugated to peroxidase. | CONJ |
| Positive Control | One (1) vial containing 1 ml of diluted positive rabbit serum. | CONTROL + |
| Negative Control | One (1) vial containing 1 ml of diluted negative human serum. | CONTROL − |
| Chromogen | One (1) bottle containing 11 ml of the chromogen tetramethylbenzidine (TMB). | SUBS TMB |
| Wash Concentrate (20X) | One (1) bottle containing 25 ml of concentrated buffer and surfactant. | WASH BUF |
| Dilution Buffer | Two (2) bottles containing 30 ml of buffered protein solution. | SPECM DIL |
| Stop Solution | One (1) bottle containing 11 ml of 0,73 M phosphoric acid. | SOLN |

**Precautions**

Do not use solutions if they precipitate or become cloudy.
Wash concentrate may show crystallization upon storage at 2-8 ºC. Crystallization will disappear after dilution to working strength.
Do not use serum that may have supported microbial growth, or is cloudy due to high lipid content. Samples high in lipids should be clarified before use.
Treat all sera as if capable of being infectious. Negative control has been tested and found negative for Hepatitis B surface antigen and for the antibody to HIV by required test methods. This product should be used under appropriate safety conditions that would be used for any potentially infectious agent.
Do not add azides to the samples or any of the reagents.

**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 bottle to 475 ml of reagent grade water. Place diluted wash buffer into a squeeze bottle with a narrow tip opening.
Note: Washings consist of filling to the top of each well, shaking out the contents and refilling.
Avoid generating bubbles in the wells during the washing steps.

**Collection and Preparation Of Serum**

Serological specimens should be collected under aseptic conditions. Coagulate blood and remove serum. Hemolysis is avoided through prompt separation of the serum from the clot.
Serum should be stored at 2 – 8 °C if it is to be analyzed within a few days.
Serum may be held for 3 to 6 months by storage at -20 ºC or lower.
Lipemic and strongly hemolytic serum should be avoided.
Do not heat inactivate serum and avoid repeated freezing and thawing of samples.

**Test samples:**

Make a **1:64** dilution of patients' sera using the dilution buffer
(e.g. 5 µl sera and 315 µl dilution buffer).

## Procedure

### Materials Provided

Entamoeba histolytica IgG ELISA Kit

### Materials Required But Not Provided

Pipettes
Squeeze bottle for washing strips (narrow tip is recommended)
Reagent grade water and graduated cylinder
Tubes for sample dilution
Absorbent paper

### Suggested Materials

ELISA plate reader with a 450 nm and a 650 to 620 nm filter (optional if results are read visually)

### Performance of Test

1. Break off number of wells needed (two for controls plus number of samples) and place in strip holder.
2. Add 100 µl (or two drops) of the negative control to well #1,
   100 µl of the positive control to well #2 and
   100 µl of the diluted (1:64) test samples to the remaining wells.

   Note: Negative and positive controls are supplied prediluted. Do not dilute further.
3. Incubate at room temperature (15 °C to 25 °C) for 10 minutes.
4. Shake out contents and wash 3 times with the diluted wash buffer.
5. Add 2 drops of Enzyme Conjugate to each well.
6. Incubate at room temperature for 5 minutes.
7. Shake out contents and wash 3 times with wash buffer. Slap wells against paper towels to remove excess moisture.
8. Add 2 drops of the Chromogen to every well.
9. Incubate at room temperature for 5 minutes.
10. Add 2 drops of the Stop Solution and mix by tapping strip holder.

**Reading of Results**

**Visually:**
Look at each well against a white background (e.g. paper towel) and record as clear or +, ++ or +++ reaction.

**ELISA Reader:**
Zero reader on air. Set for bichromatic readings at 450/650-620 nm.

**Test Limitations**

Serologic results are an aid in diagnosis but cannot be used as the sole method of diagnosis.

**Quality Control**

The use of controls allows validation of kit stability. The kit should not be used if any of the controls are out of range.
Expected values for the controls are:
**Negative** - 0.0 to 0.3 OD units
**Positive** - 0.5 OD units and above

**Troubleshooting**

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.

**Interpretation of Results**

**Interpretation of Results - ELISA Reader**

Zero ELISA reader on air. Read all wells at 450/650-620 nm.

**Positive** - Absorbance reading equal to or greater than 0.4 OD units.
**Negative** - Absorbance reading less than 0.4 OD units.

A positive OD reading indicates that the patient may be infected by *E. histolytica*.
A negative OD reading indicates that the patient has no detectable level of antibodies. This may be due to lack of infection or poor immune response by the patient.

**Interpretation of Results -Visual**

Compare results to the controls. A sample should be interpreted as positive if the degree of color is significant and obvious.

**Expected Results**

The number of individuals showing positive results can vary significantly between populations and geographic regions. If possible, each laboratory should establish an expected range for its patient population.

**Performance Data**

Study #1 – Canadian Reference Center
Compared DRG ELISA to another commercial ELISA. Found concordance of 96.3% (n=82).

Study #2 – CDC&P

| | | CDC&P | |
|---|---|---|---|
| | | + | - |
| DRG | + | 22 | 0 |
| | - | 2 | 21 |

Sensitivity of 92% (22/24)
Specificity of 100% (21/21)

**References / Literature**

1. Patterson, M. et. al. Serologic Testing for Amebiasis. *Gastroenterology*. 78:136, 1980
2. Healy, G. Laboratory Diagnosis of Amebiasis. *Bull NY Acad Med.* 47:478, 1971
3. Healy, G. Immunologic Tools in the Diagnosis of Amebiasis: Epidemiology in the United States. *Rev Infect Diseases.* Vol.8,#2:239, 1986
4. Walsh, J. Problems in Recognition and Diagnosis of Amebiasis: Estimation of the Global Magnitude of Morbidity and Mortality. *Rev Infect Diseases*. Vol.8,#2:228, 1986

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