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E. histolytica IgG ELISA

Ehisto-G-96

Intended Use

The *E. histolytica* ELISA test is a qualitative enzyme immunoassay for the detection of antibodies to *E. histolytica*, in samples of human serum or plasma. This test is intended to be performed by trained medical technologists only.

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. histolytic*

Reagents

Item	Description	Symbol
Test Strips	Microwells containing *E. histolytica* 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 2 ml of a surrogate positive control.	CONTROL +
Negative Control	One (1) vial containing 2 ml of diluted human sera.	CONTROL −
Chromogen TMB	One (1) bottle containing 11 ml of the chromogen tetramethylbenzidine (TMB).	SUBS TMB
Wash Concentrate (20X)	Two (2) bottles 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 1 M phosphoric acid.	SOLN

Statement of Warnings

- **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.
- 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 reagents and samples as potentially infectious materials. Positive control has been tested and found negative for Hepatitis B surface antigen and for the antibody to HIV be required test methods. 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.

Storage

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

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 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 Handling

Serum or plasma (collected with heparin, EDTA or citrate) should be stored at 2-8° C if it is to be analyzed within 5 days. Samples may be held for extended storage at -20° C or lower for 1 year. Freezing whole blood samples is not advised. Do not heat inactivate samples and avoid repeated freezing and thawing of samples.

Procedure

Materials Provided

E. histolytica IgG ELISA Kit

Materials Required But Not Provided

- Micropipette
- Squeeze bottle for washing strips (narrow tip is recommended)
- Reagent grade (DI) water
- Graduated Cylinder
- Sample Dilution Tubes
- Absorbent paper
- Timer

Suggested Materials

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

Proper Temperature

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

Test Procedure

Notes:

- Ensure all samples and reagents are at room temperature (15-25°C)
- 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 step should help to minimize bubbles in the wells.
- Negative and positive controls are supplied pre-diluted. DO NOT dilute further.

1. Break off number of wells needed (two for controls plus number of samples) and place in strip holder.
2. Dilute sera 1:64 using the Dilution Buffer (e.g. 5 µl sera and 315 µl dilution buffer).
3. Add **100µl** of the negative control to well #1, **100µl** of the positive control to well #2 and **100µl** of the diluted test samples to the remaining wells.
4. Incubate at room temperature for **10 minutes**, then wash.* After last wash step, slap the wells on a clean absorbent towel to remove excess wash buffer.
5. Add 100 ul of Enzyme Conjugate to each well.
6. Incubate at room temperature for **5 minutes**, then wash.* After last wash step, slap the wells on a clean absorbent towel to remove excess wash buffer.
7. Add 100 ul of the Chromogen to each well.
8. Incubate at room temperature for **5 minutes**.
9. Add 100 ul of the Stop Solution to each well. Mix wells by gently tapping the side of the strip holder with index finger for approximately **15 seconds**.
10. Read within one hour of adding Stop Solution.

* Washings consist of vigorously filling each well to overflowing and decanting contents three (3) separate times. If using automated washers; add 1 minute dwell time between washings and increase number of washes from three to five. When possible, avoid formation of bubbles in the wells as this may affect the end results.

Reading 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 450/620-650 nm.

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 0.20 OD units
Positive - 0.50 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 The Test – ELISA Reader

Zero ELISA reader on air. Read all wells at 450/650-620 nm.
Positive – Absorbance reading equal to or greater than 0.2 OD units.
Negative - Absorbance reading less than 0.2 OD units

Interpretation of The Test – Visual

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

Limitations of the Procedure

Diagnosis of *E. histolytica* infection should not be made solely based on results of the ELISA *E. histolytica* test alone, but in conjunction with other clinical signs and symptoms and other laboratory findings. Epidemiologic factors, clinical findings, exposure to endemic regions, and other laboratory results should be considered when making a diagnosis.

Expected Values

The number of antibody positive subjects in a population depends on two factors: disease prevalence and clinical criteria used to select the tested population. Because very few positives should be seen in a randomly screened population in a non-endemic area, most serology tests are not specific enough to screen non-endemic populations. Even in an endemic region, serology screening often yields many false positives if used to randomly screen patients. Serology tests are useful to test patients in an endemic region with signs and symptoms consistent with the disease.

Assay Characteristics

Study #1 – CV

Intra-assay (n=8)

Negative Samples = 5.1 %
Positive Samples = 6.6 %

Inter-assay (n=8)

Negative Samples = 6.5 %
Positive Samples = 8.0 %

Study #2 – Versus Another Commercial ELISA

		Competitors ELISA	
		+	-
Eiger ELISA	+	13	0
	-	0	45

Sensitivity of 100% (13/13)
Specificity of 100% (45/45)

References / Literature

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