

VITASSAY

Entamoeba

Rapid test for the qualitative detection of *Entamoeba histolytica* and *Entamoeba dispar* in human stool samples.

IUE-7355036 Ed01 October 2017



For professional *in vitro* diagnostic use only.

INTENDED USE

Vitassay Entamoeba is a rapid, immunochromatographic, one step assay for the qualitative detection of *Entamoeba histolytica* and *Entamoeba dispar* in human stool samples.

Simple, non-invasive and highly sensitivity screening assay to make a presumptive diagnosis of *Entamoeba* infection (amoebiasis).

INTRODUCTION

Entamoeba histolytica is a protozoan parasite that invades through the intestinal epithelium via a unique cell biologic process called trophocytosis, resulting in diarrhea, dysentery, and extra intestinal disease.

The genus *Entamoeba* contains many species, six of which are found in the human intestinal tract: *Entamoeba histolytica*, *Entamoeba dispar*, *Entamoeba moshkovskii*, *Entamoeba coli*, *Entamoeba hartmanni*, and *Entamoeba polecki*. Of these species, only *E. histolytica* is associated with pathological injuries; the others are considered to be nonpathogenic species.

Food and drink contaminated with faeces containing the cysts is a common source of infection. Most cases arise from human carriers, or cyst passers, who pass cysts in formed or semiformed stools.

Because transmission is frequently associated with contaminated food and water, young infants are not expected to develop amebiasis very often. More severe disease is associated with young age, malnutrition and immunosuppression.

The motil (trophozoite) form of *E. histolytica* inhabits the human colon where it multiplies and differentiates into cysts that are released into the environment. In turn, these cysts are responsible for transmitting the infection to another host via the fecal-oral route. The parasite invades the intestinal mucosa and causes many forms of invasive disease, including dysentery. The parasite also exhibits bloodborne spreading and causes extraintestinal lesions, mainly liver abscesses. The latter form occurs only rarely. Invasive disease occurs when virulent trophozoites disrupt the mucopithelial barrier by crossing the mucus layer, thereby damaging intestinal cells. This damage leads to inflammation and, consequently, dysentery. Nevertheless, the majority of infections seem to be asymptomatic.

PRINCIPLE

Vitassay Entamoeba is a qualitative immunochromatographic assay for the detection of *Entamoeba histolytica* and *Entamoeba dispar* in human stool samples.

The test line zone of the nitrocellulose membrane is pre-coated with monoclonal antibodies against *Entamoeba*.

During the process, the sample reacts with the antibodies against *Entamoeba*, forming conjugates. The mixture moves upward on the membrane by capillary action. If the sample is positive, antibodies present on the membrane (test line) capture the conjugate complex and a red line will be visible. Although the sample is positive or negative, the mixture continues to move across the membranes and the green control line always appears.

The presence of this green line (in the control zone (C)) indicates that sufficient volume is added; proper flow is obtained and serves as an internal control for the reagents.

PRECAUTIONS

- For professional *in vitro* use only.
- Do not use after expiration date.
- Do not use the test if its pouch is damaged.
- Specimens should be considered as potentially hazardous and handle in the same manner as an infectious agent. A new test must be used for each sample to avoid contaminations errors. Single use device.
- Tests should be discarded in a proper biohazard container after testing.
- Reagents contain preservatives. Avoid any contact with the skin or mucous membrane. Consult safety data sheet, available on request.
- Components provided in the kit are approved for use with the **Vitassay Entamoeba**. Do not use any other commercial kit component.
- Follow Good Laboratory Practices, wear protective clothing, use disposal gloves, goggles and mask. Do not eat, drink or smoke in the working area.

STORAGE AND STABILITY

Store as packaged in the sealed pouch either at refrigerated or room temperature (2-30°C/35.6-86°F) on the sealed pouch.

The test is stable until the expiration date printed on the sealed pouch.

The test must remain in the sealed pouch until use.

Do not freeze.

MATERIALS

MATERIAL PROVIDED	MATERIAL REQUIRED BUT NOT PROVIDED
<ul style="list-style-type: none"> 25 tests/kit Vitassay Entamoeba. Instructions for use. 25 vials with diluent for the dilution sample. 	<ul style="list-style-type: none"> Specimen collection container. Disposable gloves. Timer.

SPECIMEN COLLECTION

Collect sufficient quantity of feces: 1-2g or mL for liquid samples. Stool samples should be collected in clean and dry containers.

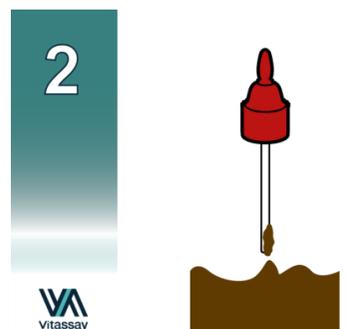
Samples can be stored in the refrigerator (2-8°C/35.6-46.4°F) for 1-2 days prior to testing. For longer storage, maximum 1 year, the specimen must be kept frozen at -20°C (-4°F). Samples must be brought to room temperature before testing.

SPECIMEN PREPARATION

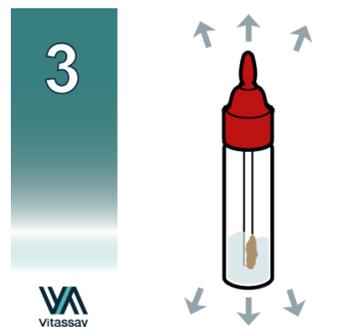
1. Remove the cap of the vial with diluent for the sample dilution (figure 1).
2. Use the stick to collect sufficient sample quantity. For solid stool, insert the stick in 4 different areas of the stool sample, taken approx. 125mg, (figure 2), and add it into the vial with diluent for the sample dilution. For liquid stool, take 125µL of the sample using a micropipette and transfer it into the vial with diluent for the sample dilution.
3. Close the vial with the diluent and stool sample. Shake vigorously the vial in order to assure good sample dispersion (figure 3).



Vial for sample dilution.



Insert the stick in 4 different areas of the stool.



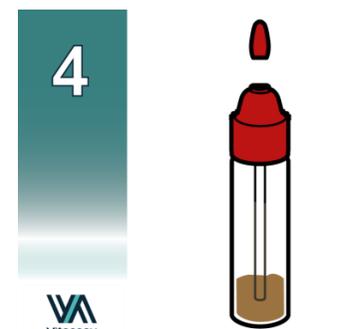
Put the sample into the vial, close the cap and shake.

PROCEDURE

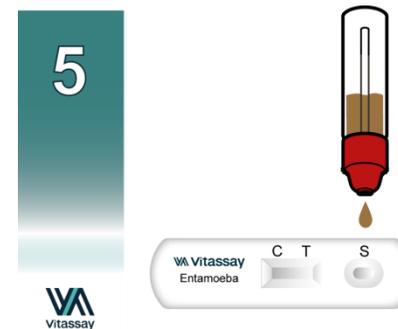
Allow the test, stool sample, controls and diluent to reach room temperature (15-30°C/59-86°F) prior to testing. Do not open pouches until the performance of the assay.

1. Shake the vial with the sample to obtain a good sample dilution.
2. Remove the **Vitassay Entamoeba** from its sealed bag just before using it.
3. Take the vial containing the diluted sample, cut the end of the cap (figure 4) and dispense 4 drops in the circular window marked with the letter S (figure 5).
4. Read the results at **10 minutes**. Do not read the results later than 10 minutes.

If the test does not run due to solid particles, stir the sample added in the sample window with the stick. If it does not work, dispense a drop of diluent until seeing the liquid running through the reaction zone.



Cut the end of the cap.



Dispense 4 drops in the circular window marked with the letter S.

INTERPRETATION OF THE RESULTS

	NEGATIVE	
	Only one green line in the control zone (C)	There is no <i>Entamoeba histolytica</i> or <i>Entamoeba dispar</i> presence. No infection caused by <i>Entamoeba histolytica</i> or <i>E. dispar</i> .
	In addition to the green line (control line C), a red line appears, (test line T)	There is presence of <i>Entamoeba histolytica</i> or <i>Entamoeba dispar</i> presence. Infection caused by <i>Entamoeba histolytica</i> or <i>Entamoeba dispar</i> .
ANY OTHER RESULTS		Invalid result, we recommend repeating the assay using the sample with another test. Note: Wrong procedural techniques or deterioration of the reagents are mostly the main reasons for control line failure. If the symptoms or situation persist, discontinue using the test kit and contact your local distributor.

Notes: The intensity of the red colored test line in the result line zone (T) will vary depending on the concentration of antigens in the specimen.

QUALITY CONTROL

Internal procedural control is included in **Vitassay Entamoeba**. Green line appearing in the results window is an internal control (C), which confirms sufficient specimen volume and correct procedural technique.

LIMITATIONS

- **Vitassay Entamoeba** must be carried out within 2 hours of opening the sealed bag.
- Only fresh or fresh-frozen unpreserved and unfixed stool samples can be tested.
- An excess of sample could cause wrong results (brown bands appear). Dilute the sample with the diluent and repeat the test.
- The intensity of test line may vary depending on the concentration of antigens.
- After one week of infection, the number of parasites in feces is decreasing, making the sample less reactive. Stool samples should be collected within one week of the onset of symptoms.
- The use of other samples different from human samples has not been established.
- The quality of **Vitassay Entamoeba** depends on the quality of the sample; Proper fecal specimens must be obtained.
- Positive results determine the presence of *Entamoeba histolytica* and/or *dispar* in fecal samples; nevertheless, a positive result should be followed up with additional laboratory techniques (biochemical method or by microscopy) to confirm the results. A confirmed infection should only be made by a physician after all clinical and laboratory findings have been evaluated and must be based in the correlation of the results with further clinical observations.
- Negative results should not be considered as conclusive; it is possible that the concentration of antigens is lower than the detection limit. If symptoms or situation still persist, a *Entamoeba histolytica* and/or *dispar* determination should be carried out with another technique (for example microscopy).

EXPECTED VALUES

Entamoeba histolytica infection is the third-greatest parasitic disease responsible for death in the world after malaria and schistosomiasis. It affects approximately 180 million people, of whom 40000 to 110000 die each year. *Entamoeba histolytica* infections are worldwide, more common in the tropics and subtropics.

Particularly exposed to this parasite are people living and traveling to the tropical and subtropical zones (Asia, Africa, India, Indonesia, Mexico, South America, South Africa). These zones climatic conditions are optimal for the Protozoan cysts, which described the external environment can survive for many days. This fact contributes to the increase in the number of infections *Entamoeba histolytica* amongst people in tropical zones.

E. histolytica differs among countries, socio-economic and sanitary conditions, and populations. It is highly endemic throughout poor and socio-economically deprived communities in the tropics and subtropics. Environmental, socio-economic, demographic, and hygiene related behavior is known to influence the transmission and distribution of intestinal parasitic infections.

PERFORMANCE CHARACTERISTICS

Clinical sensitivity and specificity

An evaluation, with faecal samples, was performed using **Vitassay Entamoeba** and these results were confirmed using another commercial qPCR test (*VIASURE Entamoeba histolytica* Real Time Detection Kit and *VIASURE Entamoeba dispar* Real Time Detection Kit, CerTest).

Results were as follows:

		VIASURE <i>Entamoeba histolytica</i> Real Time Detection Kit		
		Positive	Negative	Total
Vitassay Entamoeba	Positive	4	1	5
	Negative	1	107	108
	Total	5	108	113

Vitassay Entamoeba vs VIASURE <i>Entamoeba histolytica</i> Real Time Detection Kit		
	Mean Value E. histolytica	95% confidence interval
Sensitivity	80%	28.4-99.5%
Specificity	99%	94.9-100%
PPV	80%	28.4-99.5%
NPV	97%	94.9-100%

		VIASURE <i>Entamoeba dispar</i> Real Time Detection Kit		
		Positive	Negative	Total
Vitassay Entamoeba	Positive	15	1	16
	Negative	6	107	113
	Total	21	108	129

Vitassay Entamoeba vs VIASURE <i>Entamoeba dispar</i> Real Time Detection Kit		
	Mean Value E. dispar	95% confidence interval
Sensitivity	71%	47.8-88.7%
Specificity	99%	94.9-100%
PPV	94%	69.8-99.8%
NPV	95%	88.8-98.0%

		VIASURE <i>Entamoeba histolytica</i> Real Time Detection Kit and VIASURE <i>Entamoeba dispar</i> Real Time Detection Kit		
		Positive	Negative	Total
Vitassay Entamoeba	Positive	19	2	21
	Negative	7	214	221
	Total	26	216	242

Vitassay Entamoeba vs VIASURE <i>Entamoeba histolytica</i> Real Time Detection Kit and VIASURE <i>Entamoeba dispar</i> Real Time Detection Kit		
	Mean Value E. histolytica+ E. dispar	95% confidence interval
Sensitivity	73%	52.2-88.4%
Specificity	99%	96.7-100%
PPV	90%	69.6-98.8%
NPV	97%	93.6-98.7%

The results showed that **Vitassay Entamoeba** has a high sensitivity and specificity to detect *Entamoeba histolytica* and *Entamoeba dispar*.

Cross reactivity

No cross reactivity was detected against gastrointestinal pathogens that are occasionally present in feces:

<i>Campylobacter jejuni</i>	<i>Helicobacter pylori</i>	<i>Shigella boydii</i>
<i>Campylobacter coli</i>	<i>Listeria monocytogenes</i>	<i>Shigella dysenteriae</i>
<i>Clostridium difficile</i>	<i>Salmonella enteritidis</i>	<i>Shigella flexneri</i>
<i>Escherichia coli</i> O157:H7	<i>Salmonella paratyphi</i>	<i>Shigella sonnei</i>
<i>Cryptosporidium parvum</i>	<i>Salmonella typhi</i>	<i>Staphylococcus aureus</i>
<i>Giardia lamblia</i>	<i>Salmonella typhimurium</i>	

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SYMBOLS FOR IVD COMPONENTS AND REAGENTS

	in vitro diagnostic device		Keep dry
	Consult instructions for use		Temperature limitation
	Use by		Manufacturer
	Batch code		Contains sufficient for <n> test
DIL	Sample diluent		Catalogue number