

# VITASSAY

## Crypto+Giardia+ Entamoeba

Rapid test for the simultaneous qualitative detection of cryptosporidium, giardia, entamoeba (histolytica and dispar) in human stool samples.

IUE-7715037 Ed01 April 2017



**For professional *in vitro* diagnostic use only.**

### INTENDED USE

**Vitassay Crypto+Giardia+Entamoeba** is a rapid, immunochromatographic assay for the simultaneous qualitative detection of *Cryptosporidium*, *Giardia*, *Entamoeba (histolytica and dispar)* in human stool samples.

Simple, non-invasive and highly sensitivity immunoassay to make a presumptive diagnosis of *cryptosporidium*, *giardia* and/or *entamoeba* infection.

### INTRODUCTION

Although there could be many other causes of diarrhea, the enteric protozoa *E. histolytica*, *G. lamblia* and *Cryptosporidium spp.* have been recognized as important causes of diarrhea among human beings. *E. histolytica* is a pathogenic parasite for which humans are the primary reservoir. The clinical presentation can range from asymptomatic carriage to gastrointestinal disease and invasive disease. *E. histolytica* is morphologically identical to the nonpathogenic species *E. dispar* and *E. moshkovskii*, though genetic differences have confirmed their separation into independent species.

*G. lamblia* (synonyms: *G. intestinalis* and *G. duodenalis*) is the most common protozoan infection of the intestinal tract worldwide. Many countries, especially developing countries, show a high infection rate of giardiasis. It is believed that giardiasis is still a significant health problem. Most infected persons are children who suffer and experience growth retardation. Cryptosporidiosis is a frequent cause of diarrheal disease in humans. Infection is acquired via feco-oral route, and *C. parvum* has been recognized as the cause of large waterborne and food-borne outbreaks of gastroenteritis. Patients tend to present with a self-limiting diarrhea that may last for several weeks to months.

Transmission of intestinal parasites depends on the presence of infected individuals, poor sanitation and principally, the socioeconomic and behavioral factors in the population.

In addition, giardiasis, cryptosporidiosis and amoebiasis may result on severe diarrhea, impaired intestinal absorptive function and malnutrition, conditions often associated with poor cognitive function and failure to thrive in early childhood.

All these three parasites can cause waterborne outbreaks, and also foodborne outbreaks have been reported.

### PRINCIPLE

**Vitassay Crypto+Giardia+Entamoeba** is a qualitative immunochromatographic assay to make a presumptive diagnosis of *Cryptosporidium*, *Giardia*, *Entamoeba (histolytica and dispar)* infection.

**Strip A:** The test line zone of the nitrocellulose membrane is pre-coated with monoclonal antibodies against *Crypto*.

**Strip B:** The test line zone of the nitrocellulose membrane is pre-coated with monoclonal antibodies against *Giardia*.

**Strip C:** The test line zone of the nitrocellulose membrane is pre-coated with monoclonal antibodies against *Entamoeba (histolytica and dispar)*.

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

The presence of a **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 Crypto+Giardia+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).

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"> <li>10 tests/kit</li> <li><b>Vitassay Crypto+Giardia+Entamoeba</b></li> <li>Instructions for use.</li> <li>10 vials with diluent for sample dilution.</li> </ul> | <ul style="list-style-type: none"> <li>Specimen collection container.</li> <li>Disposable gloves.</li> <li>Timer.</li> <li>Spatula.</li> </ul> |

## SPECIMEN COLLECTION

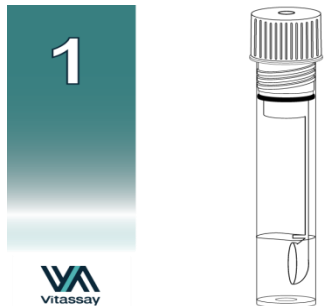
Collect sufficient quantity of feces: 1-2 g or mL for liquid samples. Stool samples should be collected in clean and dry containers.

The samples can be stored in the refrigerator (2-8°C/36-46.4°C) for 1-2 days prior to testing. For longer storage, maximum 1 year, the specimen must be kept frozen at -20°C/-4°F. The samples will be brought to room temperature before to testing.

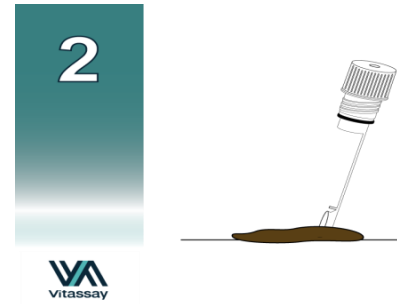
Homogenise stool sample as thoroughly as possible prior to preparation.

## SPECIMEN PREPARATION

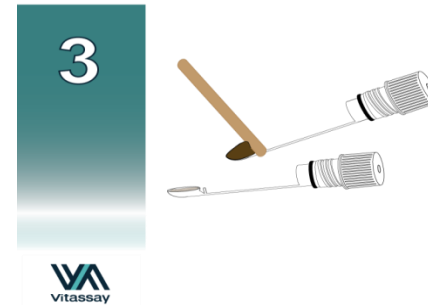
- 1 Remove the cap of the vial with diluent for sample dilution (figure 1) and use the spoon to collect sufficient sample quantity. For solid stool, insert the spoon in 4 different areas of the stool sample (figure 2), remove any excess sample with a spatula (figure 3), and place the spoon cap back into the vial for sample dilution (figure 4). For liquid stool, take a spoonful of the sample (figure 3) and transfer it into the vial for sample dilution.
2. Close the vial for sample dilution tightly and shake it to dilute and mix the sample with the diluent (figure 4).



Vial for sample dilution.



Insert the spoon in 4 different areas of the stool.



Remove excess sample with a spatula. Liquid samples: full spoon.



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 vigorously to obtain a good sample dilution.
2. Remove the **Vitassay Crypto+Giardia+Entamoeba** from its sealed bag just before using it (figure 5).
3. Take the vial for sample dilution containing the diluted sample (figure 6), place it inside the multiplex tube (figure 7). Screw the cap of the multiplex tube tightly (figure 8). The bottom of the vial

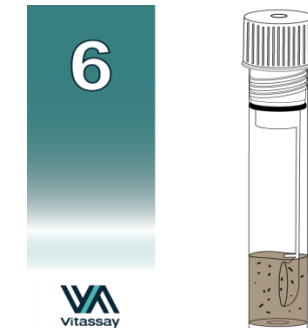
for sample dilution will break and the diluent+sample solution reaches the sample zone of the strips (figure 9).

4. Leave the multiplex tube vertically on a flat surface and read the results at **10 minutes**. Do not read the test results later than 10 minutes.

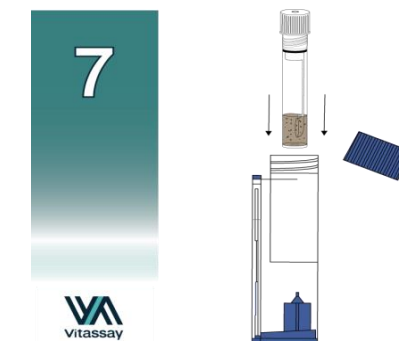
If the test does not run due to solid particles (the sample is not homogenized), migration process can stop on one or more strips. In this case, tap the end of the multiplex tube on hard surface to allow migration to start again.



Vitassay Crypto+Giardia+Entamoeba.



Vial with the diluted sample inside.



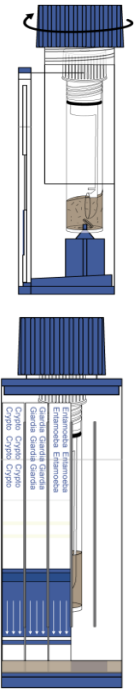
Introduce the vial with the diluted sample into the multiplex.

Introduce the vial with the diluted sample into the multiplex.

8



9



Close the cap and the bottom of the diluent vial will break.

Reaction takes place. Read results at 10 minutes.

**INTERPRETATION OF THE RESULTS**

Strip A: Crypto, Strip B:Giardia and Strip C: Entamoeba

|  |   |   |
|--|---|---|
|  | <p><b>NEGATIVE</b></p> <p>Only one green line in the control zone (C) in the three strips (A,B and C)</p>                     | <p>There is no <i>Cryptosporidium</i>, <i>Giardia</i> and <i>Entamoeba (histolytica o dispar)</i> presence.</p> |
|  | <p><b>POSITIVE</b></p> <p>In addition to the green line (control line C), a red line appears in each strip, test line (T)</p> | <p>There is <i>Cryptosporidium</i>, <i>Giardia</i> and <i>Entamoeba (histolytica o dispar)</i> presence.</p>    |

|  |  |  |
|--|--|--|
|  | <p><b>NEGATIVE</b></p> <p>Strip C (Entamoeba)→ green line</p> <p><b>POSITIVE</b></p> <p>Strip A (Crypto)→ green/red lines<br/>Strip B (Giardia)→ green/red lines</p> | <p>There is <i>Cryptosporidium</i> and <i>Giardia</i> presence. Infection caused by <i>Cryptosporidium</i> and <i>Giardia</i>.</p>   |
|  | <p><b>NEGATIVE</b></p> <p>Strip B (Giardia)→ green line</p> <p><b>POSITIVE</b></p> <p>Strip A (Crypto)→ green/red lines<br/>Strip C (Entamoeba)→ green/red lines</p> | <p>There is <i>Cryptosporidium</i> and <i>Entamoeba (histolytica o dispar)</i> presence. Infection caused by <i>Cryptosporidium</i> and <i>Entamoeba (histolytica o dispar)</i>.</p> |
|  | <p><b>NEGATIVE</b></p> <p>Strip A (Crypto)→ green line</p> <p><b>POSITIVE</b></p> <p>Strip B (Giardia)→ green/red lines<br/>Strip C (Entamoeba)→ green/red lines</p> | <p>There is <i>Giardia</i> and <i>Entamoeba (histolytica o dispar)</i> presence. Infection caused by <i>Giardia</i> and <i>Entamoeba (histolytica o dispar)</i>.</p>                 |
|  | <p><b>NEGATIVE</b></p> <p>Strip B (Giardia)→ green line<br/>Strip C (Entamoeba)→green line</p> <p><b>POSITIVE</b></p> <p>Strip A (Crypto)→ green/red lines</p>       | <p>There is <i>Cryptosporidium</i> presence. Infection caused by <i>Cryptosporidium</i>.</p>   |
|  | <p><b>NEGATIVE</b></p> <p>Strip A (Crypto)→ green line<br/>Strip C (Entamoeba)→ green line</p> <p><b>POSITIVE</b></p> <p>Strip B (Giardia)→ green/red lines</p>      | <p>There is <i>Giardia</i> presence. Infection caused by <i>Giardia</i>.</p>   |

|                          |   |   |
|--------------------------|---|---|
|                          | <p><b>NEGATIVE</b></p> <p>Strip A (Crypto)→ green line<br/>Strip B (Giardia)→ green line</p> <p><b>POSITIVE</b></p> <p>Strip C (Entamoeba)→ green/red lines</p> | <p>There is <i>entamoeba</i> presence. Infection caused by <i>Entamoeba (histolytica o dispar)</i>.</p> |
| <p>Any other results</p> | <p>Invalid results either A, B or C, we recommend repeating the assay using the same sample with another test.</p>  |   |

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

Positive results detailed in the above table should be followed up with additional confirmatory diagnostic procedures.

Single or dual simultaneous infections are more frequent than triple.

**Invalid results:** Total absence of any control coloured lines (green) indicates an invalid result, regardless of the appearance or not of the test lines (red). Wrong procedural techniques or deterioration of the reagents are mostly the main reasons for control line failure. Review the procedure and repeat the assay with a new test. If the problem persists, discontinue using the kit and contact your local distributor.

**QUALITY CONTROL**

Internal procedural controls are included in **Vitassay Crypto+Giardia+Entamoeba**. Green lines appearing in the in the results window are internal controls, which confirm sufficient specimen volume and correct procedural technique.

**LIMITATIONS**

- **Vitassay Crypto+Giardia+Entamoeba** must be carried out within 2 hours of opening the sealed bag.
- An excess of stool sample could cause wrong results (brown bands appear). Dilute the sample with the diluent and repeat the test.
- Only fresh or fresh frozen unpreserved and unfixed stool samples can be tested.
- The intensity of test line may vary depending on the concentration of antigens.
- The use of other samples different from human fecal samples has not been established.

•The quality of **Vitassay Crypto+Giardia+Entamoeba** depends on the quality of the sample; Proper faecal specimens must be obtained.

• 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 symptoms.

•Positive results determine the presence of cryptosporidium and/or giardia and/or entamoeba in fecal samples. A positive result should be followed up with additional laboratory techniques (biochemical methods, PCR, microscopy) to confirm the results. A confirmed infection should only be made by a physician after the evaluation of all clinical and laboratory findings 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 antigen is lower than the detection limit value. If symptoms or situation still persist a cryptosporidium and/or giardia and/or entamoeba determination should be carried out with another technique (for example microscopy).

#### EXPECTED VALUES

*Cryptosporidium spp.* infections occur mostly in children younger than 2 years of age, with a peak in children younger than 2 years of age. In immuno-deficient humans, especially individuals with HIV/AIDS, cryptosporidiosis can be associated with chronic, potentially life-threatening diarrhea.

In developing countries, about 200 million people have symptomatic giardiasis, with some 500000 new cases reported each year. *Cryptosporidium* accounts for up to 20% of all cases of childhood diarrhoea, whereas invasive amoebic infection by *E. histolytica* affects 50 million people worldwide each year, resulting in 40000-100000 deaths annually.

Among the main infectious diarrheagenic pathogens, *Cryptosporidium spp.* results in the most deaths among children <5 years of age.

#### PERFORMANCE CHARACTERISTICS

##### Clinical sensitivity and specificity

Several evaluations, with faecal samples, was performed using **Vitassay Crypto+Giardia+Entamoeba** and these results were compared using a microscopy technique, PCR for Crypto and Giardia strips and qPCR test (VIASURE *Entamoeba histolytica* Real Time Detection Kit and VIASURE *Entamoeba dispar* Real Time Detection Kit, CerTest) for Entamoeba strip.

Results were as follows:

|  |              | Microscopy technique/PCR |          |       |
|--|--------------|--------------------------|----------|-------|
|  |              | Positive                 | Negative | Total |
| <b>Vitassay Crypto+Giardia+Entamoeba</b> | Positive     | 25                       | 0        | 25    |
|  | Negative     | 0                        | 229      | 229   |
|  | <b>Total</b> | 25                       | 229      | 254   |
| <i>Cryptosporidium</i>                   |              |                          |          |       |

| <b>Vitassay Crypto+Giardia+Entamoeba (<i>Cryptosporidium</i>) vs Microscopy technique/PCR</b> |            |                         |
|---|------------|-------------------------|
|   | Mean Value | 95% confidence interval |
| <b>Sensitivity</b>  | >99%       | 86.3-100%               |
| <b>Specificity</b>  | >99%       | 98.4-100%               |
| <b>PPV</b>  | >99%       | 86.3-100%               |
| <b>NPV</b>  | >99%       | 98.4-100%               |

|  |              | Microscopy technique/PCR |          |       |
|--|--------------|--------------------------|----------|-------|
|  |              | Positive                 | Negative | Total |
| <b>Vitassay Crypto+Giardia+Entamoeba</b> | Positive     | 61                       | 0        | 61    |
|  | Negative     | 2                        | 191      | 193   |
|  | <b>Total</b> | 63                       | 191      | 254   |
| <i>Giardia</i>                           |              |                          |          |       |

| <b>Vitassay Crypto+Giardia+Entamoeba (<i>Giardia</i>) vs Microscopy technique/PCR</b> |            |                         |
|---|------------|-------------------------|
|   | Mean Value | 95% confidence interval |
| <b>Sensitivity</b>  | 97%        | 89.0-99.6%              |
| <b>Specificity</b>  | >99%       | 98.1-100%               |
| <b>PPV</b>  | >99%       | 94.1-100%               |
| <b>NPV</b>  | 99%        | 96.3-99.9%              |

|  |              | VIASURE <i>Entamoeba histolytica</i> Real Time Detection Kit |          |       |
|--|--------------|--|----------|-------|
|  |              | Positive   | Negative | Total |
| <b>Vitassay Crypto+Giardia+Entamoeba</b> | Positive     | 4  | 1        | 5     |
|  | Negative     | 1  | 107      | 108   |
|  | <b>Total</b> | 5  | 108      | 113   |
| <i>Entamoeba</i>                         |              |  |          |       |

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

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

| <b>Vitassay Crypto+Giardia+Entamoeba (<i>Entamoeba</i>) vs VIASURE <i>Entamoeba dispar</i> Real Time Detection Kit</b> |                      |                         |
|--|----------------------|-------------------------|
|  | Mean Value E. dispar | 95% confidence interval |
| <b>Sensitivity</b>   | 71%                  | 47.8-88.7%              |
| <b>Specificity</b>   | 99%                  | 94.9-100%               |
| <b>PPV</b>   | 94%                  | 69.8-99.8%              |
| <b>NPV</b>   | 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 |
| <b>Vitassay Crypto+Giardia+Entamoeba</b> | Positive     | 19   | 2        | 21    |
|  | Negative     | 7  | 214      | 221   |
|  | <b>Total</b> | 26   | 216      | 242   |
| <i>Entamoeba</i>                         |              |  |          |       |

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

The results showed that **Vitassay Crypto+Giardia+Entamoeba** has a high sensitivity and specificity to detect *Cryptosporidium*, *Giardia* y *Entamoeba (histolytica o dispar)*.

##### Cross reactivity

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

|   |  |                               |
|---|--|-------------------------------|
| <i>Campylobacter jejuni</i>                   | <i>Giardia lamblia</i> (Strip A and C) | <i>Salmonella typhimurium</i> |
| <i>Campylobacter coli</i>                     | <i>Helicobacter pylori</i>             | <i>Shigella boydii</i>        |
| <i>Clostridium difficile</i>                  | <i>Listeria monocytogenes</i>          | <i>Shigella dysenteriae</i>   |
| <i>Cryptosporidium parvum</i> (Strip B and C) | <i>Salmonella enteritidis</i>          | <i>Shigella flexneri</i>      |
| <i>Escherichia coli</i> O157:H7               | <i>Salmonella paratyphi</i>            | <i>Shigella sonnei</i>        |
| <i>Entamoeba histolytica</i> (Strip A and B)  | <i>Salmonella typhi</i>                | <i>Staphylococcus aureus</i>  |










## REFERENCES

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3. MARIT G. TELLEVIK; SABRINA J. MOYO; BJORN BLOMBERG; TORUNN HJOLLO, SAMUEL y. MASELLE; NINA LANGELAND; KURT HANEVIK. "Prevalence of Cryptosporidium parvum/hominis, Entamoeba histolytica and Giardia lamblia among Young Children with and without Diarrhea in Dar es Salaam, Tanzania". PLoS Negl Trop Dis 9(10): e0004125, October 2015.

## 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                 |



