

VITASSAY

Crypto

Rapid test for the qualitative detection of cryptosporidium in human stool samples.

IUE-7355033 Ed00 April 2016



For professional *in vitro* diagnostic use only.

INTENDED USE

Vitassay Crypto is a rapid one step immunochromatographic assay for the qualitative detection of cryptosporidium in human stool samples.

Simple, non-invasive and highly sensitive screening assay to make a presumptive diagnostic of a possible cryptosporidiosis.

INTRODUCTION

Intestinal parasitic infections remain a serious public health problem globally. They have been associated with human malnutrition. Intestinal parasites are organisms that live in the gastrointestinal tract of humans and animals; they are the common cause of human diarrheal disease worldwide leading to significant morbidity and mortality in the world, particularly in developing countries.

Lack of safe drinking water and environmental sanitation are largely responsible for more cases of diarrheal diseases in many developing countries every year. Although there could be many other causes of diarrhea, the enteric protozoa *Cryptosporidium parvum* have been recognized as important causes of both outbreak-related and sporadic diarrhea in humans.

Symptoms on human cryptosporidiosis include diarrhea abdominal pain, nausea or vomiting and low grade fever. These are usually self-limiting, albeit after 2 or even 3 weeks, but can be prolonged or invasive and life-threatening in patients with severe T-cell immune-deficiency.

Transmission of *Cryptosporidium* is mainly through fecal-oral route, as well as through contaminated water and food, person-to-person spread and contact with infected animals.

PRINCIPLE

Vitassay Crypto is a qualitative immunochromatographic assay for the detection of cryptosporidium in human stool samples.

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

During the process, the sample reacts with the antibodies against crypto, 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.
- 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**. 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.

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 Crypto.• Instructions for use.• 25 Vials with diluent for the sample dilution.	<ul style="list-style-type: none">• Specimen collection container.• Disposable gloves.• Timer.

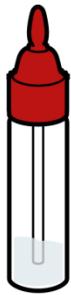
SPECIMEN COLLECTION

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

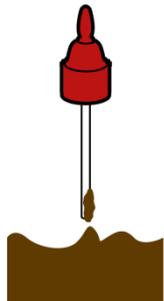
Samples can be stored in the refrigerator (2-8°C/35.6-46.4°F) for 1-2 days prior to testing. For longer storage, maximum 1 year, the specimen must be kept frozen at -20°C (-4°F). Samples must be brought to room temperature before testing.

SPECIMEN PREPARATION

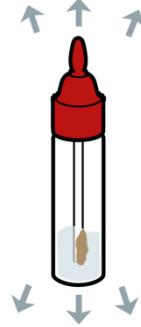
1. Remove the cap of the vial with diluent sample dilution (figure 1).
2. Use the stick to collect sufficient sample quantity. For solid stool, insert the stick in 4 different areas of the stool sample, taken approx. 125mg, (figure 2), and add it into the vial with diluent for the sample dilution. For 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.



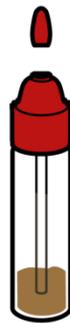
Put the sample into the vial, close the cap and shake.

PROCEDURE

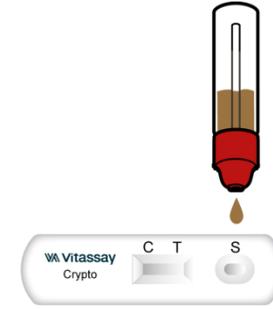
Allow the test, stool sample, controls and diluents to reach room temperature (15-30°C/59-86°F) prior to testing. Do not open pouches until the performance of the assay.

1. Shake the vial with sample to obtain a good sample dilution.
2. Remove the **Vitassay Crypto** 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	There is no cryptosporidium presence. No infection caused by cryptosporidium.
		Only one green line in the control zone (C).	
		POSITIVE	There is presence of cryptosporidium. Infection caused by cryptosporidium.
		In addition to the green line (control line C), a red line appears (test line T).	
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 Crypto**. **Green** line appearing in the results window is an internal control, which confirms sufficient specimen volume and correct procedural technique.

LIMITATIONS

- **Vitassay Crypto** 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 Crypto** depends on the quality of the sample; Proper fecal specimens must be obtained.
- Positive results determine the presence of *Cryptosporidium* 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 *Cryptosporidium* determination should be carried out with another technique (e.g. microscopy).

EXPECTED VALUES

Zoonotic pathogens are responsible for about 75% of the new diseases affecting humans. *Cryptosporidium* is a globally distributed protozoan parasite infecting humans, domestic animals, and many wild vertebrates.

Cryptosporidium spp. are also increasingly recognised as an important cause as an important cause of childhood morbidity and mortality in developing countries.

In developing countries where there is low hygiene level, poor sanitation, no good water management, and frequent contact with animals, the burden of cryptosporidiosis remains to be a major health problem.

PERFORMANCE CHARACTERISTICS

Clinical sensitivity and specificity

An evaluation, with fecal samples, was performed using **Vitassay Crypto** and these results were confirmed using a microscopy technique and PCR (positive results).

Results were as follows:

		Microscopy technique/PCR		
		Positive	Negative	Total
Vitassay Crypto	Positive	25	0	25
	Negative	0	229	229
	Total	25	229	254

Vitassay Crypto vs Microscopy technique/PCR			
Sensitivity	Specificity	PPV	NPV
>99%	>99%	>99%	>99%

The results showed that **Vitassay Crypto** has a high sensitivity and specificity to detect *Cryptosporidium*.

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>	<i>Salmonella paratyphi</i>	<i>Shigella sonnei</i>
<i>Entamoeba histolytica</i>	<i>Salmonella typhi</i>	<i>Staphylococcus aureus</i>
<i>Giardia lamblia</i>	<i>Salmonella typhimurium</i>	

REFERENCES

1. DR. PARUL PATEL; DR. SACHIN PATEL; DR. NIDHI SOOD; DR. PALAK RAO; DAYA RADADIYA. "A study of the prevalence of *Cryptosporidium Parvum* in stool samples of patients of Tertiary Care Hospital, Ahmedabad". National Journal of Integrated Research in Medicine 2015; Vol. 6(4) July-August, pp. 91-93.
2. STEPHEN J. HADFIELD; JUSTIN A. PACHEBAT; MARTIN T. SWAIN; GUY ROBINSON; SIMON JS CAMERON; JENNA ALEXANDER; MATTHEW J. HEGARTY; KRISTIN ELWIN; RACHEL M. CHALMERS. "Generation of whole genome sequences of new *Cryptosporidium parvum* isolates directly from stool samples". BioMed Central Genomics (2015) 16:650.
3. TECHALEW SHIMELIS; ENDALE TADESSE. "Performance evaluation of point-of-care test for detection of *Cryptosporidium* stool antigen in children and HIV infected adults". Parasites and Vectors 2014, 7:227.

4. MARTIN KVÁC; KAMILA SAKOVÁ; DANA KVETONOVÁ; MARTA KICIA; MARIA WESOLOWSKA; JOHN MCEVOY; BOHUMIL SAK. "Gastroenteritis caused by the *Cryptosporidium* hedgehog genotype in an immunocompetent man". Journal of Clinical Microbiology, January 2014, Volume 52, Number 1, pp. 347-349.

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
	Sample diluent		Catalogue number



