

**INTENDED USE**

The Proflow™ Crypto-Giardia Combi test is a single use rapid membrane immunoassay for the qualitative detection of Cryptosporidium and Giardia antigens in human faecal samples to aid in the diagnosis of cryptosporidiosis and giardiasis. For *In Vitro* Diagnostic Use.

**SUMMARY AND EXPLANATION**

Cryptosporidiosis is a diarrhoeal disease caused by microscopic parasites of the genus *Cryptosporidium*. Once an animal or person is infected, the parasite lives in the intestine and passes in the faeces. The parasite is protected by an outer shell that allows it to survive outside the body for long periods of time and makes it very resistant to chlorine-based disinfectants. Giardiasis is a diarrhoeal illness seen throughout the world. It is caused by a flagellate protozoan parasite, *Giardia intestinalis*, also known as *G. lamblia* and *G. duodenalis*. Giardia is a common cause of gastrointestinal disturbance. The incidence of Giardia is generally higher in countries where access to clean water and basic sanitation is lacking. Nearly all children in this setting will acquire Giardia at some point in their childhood, and the prevalence of the parasite in young children can be as high as 10%-30%. In areas such as Western Europe and the United States of America, Giardia infection is associated with ingestion of contaminated water, person-to-person spread, recent foreign travel, and recreational swimming.

**PRINCIPLE OF THE TEST**

The Proflow™ Crypto-Giardia Combi test is a single use rapid membrane immunoassay for the qualitative detection of Cryptosporidium and Giardia antigens in human faeces. Specific antibodies to Cryptosporidium and Giardia antigens are coated onto the test line region of the strip. During testing the sample migrates along the membrane by capillary action and is allowed to react with the conjugate on the test strip. In the case of a positive result the specific antibodies present on the membrane will capture the coloured conjugate. A green line should always appear in the control line region to show that sufficient volume was added, proper flow was obtained and the reagents functioned correctly. Failure of the control line to appear, whether or not test lines are present, indicates an invalid assay. The test is interpreted by the presence or absence of visibly detectable coloured lines in the test region at 10 minutes or less depending on the concentration of antigen present. A positive Cryptosporidium result will show a pink/red test line and a green control line, indicating that Cryptosporidium antigen is present in the sample. A positive Giardia result will show a pink/red test line and a green control line, indicating that Giardia antigen is present in the sample. A negative test result, read at 10 minutes, will show only a green control line, indicating that Cryptosporidium and Giardia antigens were not detectable in the sample.

**MATERIALS PROVIDED**

- PL.3401 Proflow™ Crypto-Giardia Combi Test Devices: 20 devices
- PL.3402 Proflow™ Crypto-Giardia Combi Sample Prep Device: 20 devices
- Package insert

**MATERIALS REQUIRED BUT NOT PROVIDED**

- Sample collection container
- Timer or stopwatch
- Biohazard disposal container
- Disposable gloves

**STABILITY AND STORAGE**

- Store all components at 2-30°C.
- Do not freeze or overheat.
- Do not use beyond the expiration date printed on the outer package label.
- The test kits should be kept away from direct sunlight, moisture and heat.

**PRECAUTIONS**

- For *In Vitro* Diagnostic Use only.
- For professional use only.
- Directions should be read and followed carefully.
- Tests are for single use only. Do not reuse.
- Reagents are provided at the necessary working strength. Do not dilute reagents.
- Do not interchange reagents between kits with different lot numbers.
- Do not use kits or reagents beyond the stated expiration dates.
- Microbial contamination of reagents may decrease the accuracy of the assay.
- Treat all materials as if they were infectious and dispose of all material in accordance with local regulation.

**SAMPLE STORAGE AND COLLECTION**

- Collect sufficient quantity of faeces (1-2g or mL for liquid samples)
- Faecal samples should be collected in clean and dry containers (no preservatives or transport media).
- Samples may be stored at 2-8°C for 1-2 days or at -20°C for longer periods before testing.
- Allow all samples to equilibrate to room temperature before testing.
- Freezing and thawing cycles are not recommended.
- Make sure that samples are not treated with solutions containing formaldehyde or its derivatives.

**TEST PROCEDURE**

Use a separate sample collection vial and test for each sample or control. Allow the tests, faeces samples and buffer to reach room temperature prior to testing.

1. Introduce the swab or stick four times into the faeces up to the thread of the stick to pick up approx. 50 mg, and put back into the sample preparation device with buffer. For liquid faeces samples, aspirate with a dropper and add 50 µL into the sample preparation device.
2. Shake the sample preparation device to ensure good sample dispersion.
3. Remove the Proflow™ Crypto-Giardia Combi test from its sealed pouch.
4. Break off the top of the vial on the sample preparation device.
5. Dispense 3 drops (100 µL) into the sample well on the test (S).
6. Read the result at 10 minutes.

**QUALITY CONTROL PROCEDURE**

It is recommended that a positive and negative control be used to ensure that the test is performing as expected. The control line (C) is a procedural control and will show that the test has been performed correctly; proper flow occurred and that the test reagents functioned as expected. When a green line appears at the control line position this indicates the test has been performed correctly. The control line will appear on all valid tests, whether or not the sample is reactive or non-reactive (refer to the Interpretation of Results section).

**INTERPRETATION OF RESULTS**
**Positive Cryptosporidium**

One pink/red line of any intensity will appear at test line 1; a green line will appear at the control line. This indicates a reactive result that is interpreted as positive for Cryptosporidium antigen.

**Positive Giardia**

One pink/red line of any intensity will appear at test line 2; a green line will appear at the control line. This indicates a reactive result that is interpreted as positive for Giardia antigen.

**Positive Cryptosporidium and Giardia**

Two pink/red lines of any intensity will appear at test lines 1 and 2; a green line will appear at the control line. This indicates a reactive result that is interpreted as positive for both Cryptosporidium and Giardia antigens.

**Negative**

A single green line of any intensity appears in the test window at the control line position. There are no lines at the test line positions. This indicates a non-reactive result that is interpreted as negative for both Cryptosporidium and Giardia antigens.

**Positive Crypto**


Test line 1  
Control line  
Test line 2

**Positive Giardia**

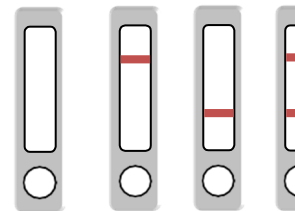
**Positive Crypto and Giardia**


Test line 1  
Control line  
Test line 2

**Negative Crypto and Giardia**

**Invalid**

No line appears in the test window at the control line position. This is an invalid result and cannot be interpreted. This is irrespective of whether or not pink/red lines appear in the test window at the test line positions. If either condition below occurs, the test should be repeated with a new test.



Test line 1  
Control line not visible  
Test line 2

## LIMITATIONS OF THE PROCEDURE

- The Proflow™ Crypto-Giardia Combi test will only indicate the presence of parasites in the sample (qualitative detection) and should be used for the detection of Cryptosporidium and Giardia antigens in faeces samples only. Neither the quantitative value nor the rate of increase in antigen concentration can be determined by this test.
- An excess of sample could cause wrong results (brown bands appear). Dilute the sample with the buffer and repeat the test.
- Do not use samples treated with solutions containing formaldehyde or its derivatives.
- The test must be carried out within 2 hours of opening the sealed bag.
- Mucous and/or bloody faecal samples can cause non-specific reactions. A positive result with a mucous or bloody sample should be confirmed with other techniques.
- If the test result is negative and clinical symptoms persist, additional testing using other clinical methods is recommended. A negative result does not at any time preclude the possibility of cryptosporidiosis or giardiasis.
- After one week of infection, the number of parasites in faeces falls, making the sample less reactive. Faeces samples should be collected within one week of the onset of symptoms.
- This test provides a presumptive diagnosis of cryptosporidiosis and/or giardiasis. All results must be interpreted together with other clinical information and laboratory findings available to the clinician.

## PERFORMANCE CHARACTERISTICS

### EXPECTED VALUES

Cryptosporidium has caused several large waterborne disease outbreaks of gastrointestinal illness, with symptoms including diarrhoea, nausea and stomach cramps. People who are severely immunocompromised are likely to have more severe and persistent symptoms than healthy individuals.

Giardia is prevalent throughout the world, including temperate, high-income countries such as the UK and the US. Several studies have examined the spread of giardiasis in international travellers.

### SENSITIVITY AND SPECIFICITY

An evaluation was performed against another commercially available rapid test. Results were confirmed by a qPCR technique (VIASURE *Cryptosporidium*, *Giardia* & *E. histolytica* Real Time Detection Kits, CerTest Biotec), and are as follows:

	Proflow™ Crypto-giardia kit vs. competitor kit (Crypto line)		Proflow™ Crypto-Giardia kit vs. competitor kit (Giardia line)	
		95% CI (confidence interval)		95% CI (confidence interval)
<b>Sensitivity</b>	94.3%	80.8-99.3%	95.6%	84.9-99.5%
<b>Specificity</b>	100.0%	96.0-100.0%	97.5%	91.4-99.7%
<b>PPV</b>	100.0%	89.4-100.0%	95.6%	84.9-99.5%
<b>NPV</b>	97.8%	92.3-99.7%	97.5%	91.4-99.7%










### CROSS-REACTIVITY

An evaluation was performed to determine the cross-reactivity of the Proflow™ Crypto-Giardia Combi test. There was no cross-reactivity with common intestinal pathogens, other organisms and substances occasionally present in faeces:

- Adenovirus
- Astrovirus
- Calprotectin
- *Campylobacter jejuni*
- *Clostridium difficile* Ag GDH / Toxin A / B
- *Clostridium perfringens*
- *Cryptosporidium parvum* (Giardia line)
- *Entamoeba histolytica*
- *Escherichia coli* O:111 / O:026 / O157
- *Giardia a-1 giardin* / CWP1 (Crypto line)
- *Helicobacter pylori*
- Haemoglobin (human / pig / bovine)
- Lactoferrin (human)
- Legionella
- *Listeria monocytogenes*
- Norovirus G1 / GII
- Rotavirus
- *Salmonella enteritidis / paratyphi / typhi / typhimurium*
- *Shigella boydii / dysenteriae / flexneri / sonnei*
- *Streptococcus pneumococcal / pyogenes*
- Transferrin (human)
- *Yersinia enterocolitica* O:3 / O:9

### REFERENCES

- Hill D.R. and Nash T.E. Intestinal Flagellate and Ciliate Infections. In: Guerrant RL, Walker D.H. and Weller P.F. eds. Tropical Infectious Diseases. *Principles, Pathogens & Practice*. 2nd ed. Elsevier, Philadelphia. 2006:984-8.
- Copue S., Delabre K., Pouillot R. et al. Detection of Cryptosporidium, Giardia and Enterocytozoon bienuei in surface water, including recreational areas: a one year prospective study: *FEMS Immunol Med Microbiol*. 2006;47:351-9.
- Stuart J.M., Orr H.J., Warburton F.G. et al. Risk Factors for Sporadic Giardiasis: A Case-Control Study in Southwestern England. *Emerg. Infect Dis*. 2003; 9, 2

	= Use by
	= Lot number
	= Catalogue number
	= Manufacturer
	= Authorized Representative in the European Community
	= Contains sufficient for <n> tests
	= In vitro diagnostic medical device
	= Temperature limitation
	= Consult instructions for use

**EC REP** Advena Ltd. Tower Business Centre, 2<sup>nd</sup> Floor,  
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IC test: Proflow™ Crypto-Giardia kit (Crypto line)	Competitor kit (Crypto line)		
	Positive	Negative	Total
	Positive	33	0
Negative	2	91	93
Total	35	91	126

IC test: Proflow™ Crypto-Giardia kit (Giardia line)	Competitor kit (Giardia line)		
	Positive	Negative	Total
	Positive	43	2
Negative	2	79	81
Total	45	81	126

