CRYPTO+GIARDIA CARD

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

In vitro immunochromatographic test for qualitative detection of Cryptosporidium and Giardia antigens in human faeces specimens, with positive and negative control

I. INTRODUCTION AND INTENDED USE

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 stool. 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. Both the disease and the parasite are commonly known as "Crypto." **The test is specific for Cryptosporidium parvum**.

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 in both high- and low-income countries. Giardia may be a cause of 2%-5% of cases of diarrhoea in high-income countries. Crypto + Giardia CARD is a immunochromatographic test for qualitative detection of Cryptosporidium and Giardia antigens in human faecal specimens.

II. PRINCIPLE OF THE TEST

The membrane is pre-coated with antibodies against Cryptosporidium and Giardia antigens on the test line region. During testing, the sample reacts with the particle coated with anti-Cryptosporidium antibodies and with anti-Giardia antibodies which were pre-dried on the test strip. The mixture moves upward on the membrane by capillary action. In the case of any positive result the specific antibodies present on the membrane will react with the mixture conjugate and generate one or two coloured lines. A green coloured band always appears in the control line and serves as verification that sufficient volume was added, that proper flow was obtained and as an internal control for the reagents.

III. REAGENTS AND MATERIALS

Each kit contains:

Crypto+Giardia Card (25 items) cassettes packaged with a desiccant in individual aluminum pouches

Diluent –Extraction buffer (25x1.0 mL tubes) Sample Diluent of buffer containing proteins and preservative

Positive Control: N.1 vial with dropper containing non-infectious components, sodium azide (NaN_3) as preservative (1 x 0,5 mL).

Negative Control: use Extraction buffer-Diluent

Dropper 10 items

Instruction for use (1)

Required materials (not supplied)

Specimen collection container; Disposable gloves; Timer; Testing tube/vial; Dropper

IV. SPECIAL PRECAUTIONS

- All operations linked to the use of the test must be performed in accordance with Good Laboratory Practices.
- The kit is for professional use and for in vitro diagnosis only.
- Avoid touching the nitrocellulose strip.
- Use disposable gloves during sample manipulation.
- All the specimens should be considered potentially hazardous and handled in the same manner as an infectious agent.

Always perform the enrichment of the sample. Do not use directly on stool samples. A lack of enrichment could produce unreliable results.

- Do not use reagents from other kits
- Discard the diluent if contaminated
- Do not use after expiration date. Do not use the test if pouch is damaged.

V.STORAGE AND STABILITY

Store as packaged in the sealed pouch either at refrigerated or room temperature (2-30ºC/36-86ºF). The test is stable through the expiration date printed on the sealed pouch. The test must remain in the sealed pouch until use. Do not freeze.

VI. SPECIMENS COLLECTION

Collect sufficient quantity of faeces (1-2 g or mL for liquid sample). Stool samples should be collected in clean and dry containers (no preservatives). It this is not possible, to use fixed samples or stored in medium like modified Cary-Blair or Stuart. The samples can be stored in the refrigerator **(2-4ºC/36-40ºF) for 4-5 days prior to testing**. For longer storage the specimen must be kept frozen at –20ºC/4ºF. In this case, the sample will be totally thawed, and brought to room temperature before testing.

Liquid or Semi-Solid Stools

Using a separate pipette (included with the kit) for each stool, draw stool of the sample itself. Dispense 6-7 drops of each stool into a separate extraction tube. Mix carefully, then vortex 15 seconds.

Care should be taken when pipetting semi-solid stool. The addition of less than indicated of stool may cause a false-negative test. The addition of more than indicated of stool may cause invalid results due to restricted sample flow.

Formed / Solid Stools

Unscrew the top of the extraction tube. Collect the stool sample with the tip of the collection device by dipping in three different places of the same stool specimen.. Verify to transfer a small portion (approximately 6 mm diameter) of stool. Put the collection device back into the plastic test tube. Shake the extraction tube in order to get an homogeneous solution. Wait at least 3 minutes. Repeat the operations just to obtain a dark yellow-brown solution, if necessary.

The transfer of too little stool, or failure to mix and suspend the stool in extraction tube completely may result in a false-negative test results. Care should be taken to transfer no less and no more than the amount indicated. The sample should be thoroughly mixed with a vortex before testing. The addition of excessive amount of stool may cause invalid results due to restricted sample flow.

VII. PROCEDURES FOR TEST

Allow the tests, samples and buffers to reach to room temperature (15-30ºC/59-86ºF) prior to testing. Do not open the pack until ready to perform the assay.

1. Remove the Card from its sealed pouch and use it as soon as possible.
2. Mix gently the stool collection tube. Mix 15 seconds with vortex. Cut the end of the cap.
3. Dispense 2-3 drops of enriched sample (about 100 µL) into the specimen well (S). Start the timer.
4. Read the result at **10 minutes** after dispensing the sample (do not read after 15 minutes)
5. Interpreting the results according the scheme in paragraph VIII.

Procedure for the controls

Add 2-3 drops (100 µL) of **Positive**/**Negative control** into the specimen well (S) and read the result at 10 minutes.

VIII. INTERPRETING THE RESULTS

The results must be interpreted in the following way:

CRYPTO **GIARDIA** **CRYPTO-GIARDIA** **NEGATIVE** **INVALID**

CRYPTO POSITIVE: two bands appears across the central window: one **red** in the result region and one **green** in the control region.
GIARDIA POSITIVE: two bands appears across the central window: one **blue** in the result region and one **green** in the control region.
CRIPTO-GIARDIA POSITIVE: three bands appears across the central window: one **red** and one **blue** in the result region and one **green** in the control region.
NEGATIVE: only one **green** band appears across the central window (control line) in the control region.
INVALID: total absence of any band or appearance of band/s red and/or blue (absence of the green band).
Note: Insufficient specimen volume, incorrect procedural techniques or deterioration of the reagents are the most likely reasons for control line failure.

INTERPRETATION OF RESULTS FOR CONTROLS

NEGATIVE CONTROL: only one green band appears across the central window (control line) in the control region.
POSITIVE CONTROL: three bands appears across the central window: two purple in the result region and one green in the control region.

NOTE ON THE INTERPRETATION OF RESULTS

The intensity of the red and blue coloured bands in the result line region will vary depending on the concentration of antigens in the specimen. However, neither the quantitative value, nor the rate of increase in antigens can be determined by this qualitative test.

IX. PERFORMANCE

Sensitivity e Specificity

Some faecal champions have been studied by means of microscopic examination/PCR and with Crypto+Giardia Card showing:

Cryptosporidium results:

		Microscopy Technique/PCR		
		+	-	
Crypto+Giardia CARD	+	25	0	25
	-	0	229	229
		25	229	254
		Sensitivity	Specificity	
		>99%	>99%	

PPV	NPV
>99%	>98%

Giardia results:

		Microscopy Technique/PCR		
		+	-	
Crypto+Giardia CARD	+	61	0	61
	-	2	191	193
		63	191	254
		Sensitivity	Specificity	
		97%	>99%	

PPV	NPV
>99%	>99%

X. CROSS-REACTIVITY

It was performed an evaluation to determine the cross reactivity of Crypto-Giardia card. There is not cross reactivity with common gastrointestinal parasites occasionally present in faeces like: Acinetobacter lwoffi, Campylobacter jejuni, Aeromonas hydrophila, E. coli 0157:H7, Salmonella typhimurium, Salmonella enteritidis, Enterobacter cloacae, Klebsiella pneumoniae, Pseudomonas aeruginosa, Proteus mirabilis, Serratia marcescens, Shigella flexneri, Stenotrophomonas maltophilia, Helicobacter pylori, Yersinia enterolitica 01, Yersinia enterolitica 03, Yersinia enterolitica 09, Rotavirus, Adenovirus (A to F groups), Adenovirus 40/41, Campylobacter coli, E. coli 0117:H7, E. coli O55:H7, E. coli O157 VT neg (EH431), E. coli O157 VT neg (EH546), E. coli O157:H19, E. coli O7:H1, E. coli O116:H-, E. coli K99, Cryptosporidium parvum, Escherichia hermanii, Entamoeba hystolitica,Brucella melitensis, Brucella abortus.

XI. LIMITS OF THE KIT

- Crypto+Giardia Card will only indicate the presence of parasites in the specimen (qualitative detection) and should be used for the detection of Cryptosporidium and Giardia antigens in faeces specimens 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 specimens treated with solutions containing formaldehyde or its derivatives.
- 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 is decreasing, making the sample less reactive. Stool 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 physician.

XII. REFERENCES

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

	In Vitro Diagnostic Medical Device		Temperature limitation	LOT	Batch code (EXXX)		Manufacturer		Keep dry	NON STERILE	Non-sterile
	Consult Instructions for use		Use by (year/month)	REF	Catalogue number		Do not reuse		Fragile, handle with care		Keep away from heat

CONTENT (25 tests) **COD. VC1023**

Crypto+Giardia CARD	25 Devices (Card)
Extraction Buffer-Diluent	25 x 1mL
Positive Control	1 x 0.5 mL
Dropper	10 items
Instruction for use	1 item

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