INSTRUCTIONS FOR USE

CRYPTO+GIARDIA CARD PLUS

***IN VITRO* IMMUNOCHROMATOGRAPHIC TEST FOR QUALITATIVE DETECTION OF *CRYPTOSPORIDIUM* AND *GIARDIA* ANTIGENS IN HUMAN FAECES SPECIMENS, INCLUDING CONTROLS**

1 – INTRODUCTION AND INTENDED USE

For ***in Vitro*** diagnostic use only

Giardia and *Cryptosporidium* are parasites that can be found in water. *Giardia* causes an intestinal illness called giardiasis. *Cryptosporidium* is responsible for a similar illness called cryptosporidiosis. These infections have become the most common causes of waterborne diseases (found in both drinking and recreational water) in humans.

Giardia, a flagellated protozoan, inhabits the upper part of the small intestine of its host and has a two major states in the life cycle: trophozoites which produces the antigens (α-1 giardin) and cyst with produces the antigens (CWP1). After the host ingest the cysts, which are the infective stage, the trophozoites emerge from the cysts in the duodenum and attach to the small intestinal mucosa. They undergo mitotic division in the intracellular lumen, some will encyst to protect themselves and will be eliminated from the host in the feces. The trophozoite is the vegetative form and replicates in the small intestine. Giardiasis is a diarrheal illness caused by a very small parasite, *Giardia intestinalis* (also known as *Giardia lamblia* and *Giardia duodenalis*).

Once an animal or person is infected with *Giardia*, the parasite lives in the intestine and is passed in the stool. The parasite is protected by an outer shell and can survive outside the body and in the environment for a long time. The most common symptoms of giardiasis include: diarrhea, loose or watery stool, stomach cramps and upset stomach. These symptoms generally begin 1-2 weeks after infection, and may last 2-6 weeks in healthy individuals. Sometimes symptoms last longer and may lead to weight loss and dehydration. Some people will have no symptoms. However, people with weakened immune systems (e.g., persons with HIV/AIDS, cancer patients, and transplant patients) or the elderly may have a more serious infection that can lead to severe illness or death.

Cryptosporidium parvum is the major cause of persistent diarrhoea in developing countries. This parasite is recognized as a highly infectious enteric pathogen and infective stage is transmitted by the fecal-oral route. Symptoms of cryptosporidiosis include watery diarrhoea, stomach cramps, weight loss, nausea and sometimes fever.

CRYPTO+GIARDIA CARD PLUS is a immunochromatographic, manual assay for the simultaneous qualitative detection of Cryptosporidium and Giardia (α1-giardin and/or CWP1) in stool samples. The test offers a simple and highly sensitive screening assay to make a presumptive diagnosis of cryptosporidiosis and/or giardiasis. In the kit is included a positive control.

2 - PRINCIPLE OF THE METHOD

Crypto+Giardia Card Plus is an non-invasive, simple to perform, rapid and very accurate immunochromatographic method for the determination of Crypto and Giardia (α1-giardin and CWP1) antigens in stool samples.

The strip consists of a nitrocellulose membrane pre-coated with mouse monoclonal antibodies against Giardia on the first test line (T), in the results window, and against Cryptosporidium on the second test line (T) and with rabbit polyclonal antibodies, on the control line (C), against a specific protein.

The label/sample absorbent pad is sprayed with test label solution (mouse monoclonal antibodies anti-Giardia) conjugated to blue polystyrene latex; (mouse monoclonal antibodies anti-Cryptosporidium) conjugated to red polystyrene latex and control label solution (specific binding protein) conjugated to green polystyrene latex, forming coloured conjugate complexes.

If the sample is Giardia positive, the antigen of the diluted sample reacts with the blue-coloured conjugate complex (anti-Giardia monoclonal antibodies-blue polystyrene microspheres) which was previously pre-dried on the absorbent pad. If the sample is Cryptosporidium positive, the antigen of the diluted sample reacts with the red-coloured conjugate complex (anti-Cryptosporidium monoclonal antibodies-red polystyrene microspheres) which was previously pre-dried on the absorbent pad. The mixture then moves upward on the membrane by capillary action. As the sample flows through the test membrane, the binding conjugate complexes migrate. The anti-Giardia and/or anti-Cryptosporidium antibodies present on the membrane (test lines) capture the coloured conjugate and the blue and/or red line will be visible. These bands is used to interpret the result.

If the sample is negative, there is no Giardia and Cryptosporidium antigen presence and yet, the antigen may be present in a concentration lower than the detection limit value, for which the reaction will not take place with the blue and red-coloured conjugate complex. The anti-Giardia and anti-Cryptosporidium antibodies present on the membrane (test lines) will not capture the antigen-red/blue-coloured conjugate complexes (not formed), for which the blue and red lines will not appear. Whether the sample is positive or not the mixture continues to move across the membranes to the immobilized specific antibodies placed in the control lines. The anti-specific protein antibodies present on both membranes will capture control green-conjugate complex and both control lines will always appear. The presence of these green lines serve as: 1) verification that sufficient volume is added, 2) that proper flow is obtained and 3) an internal control for the reagents.

3 - MATERIALS PROVIDED – PACKAGING

Product	Type	REF	Pack
Crypto+Giardia Card Plus CND: W0104050299; EDMA: 15.05.10.90; RDM: 1671716/R	Immunochromatographic test	**VC1023P** (25 tests)	25 sealed in foil pouch containing the device, with dessicant. 25 plastic tubes with dropper tip containing the extraction liquid; to use also like negative control (25 x 1 mL) 1 glass dropper bottle containing Positive Control: mixture with non infectious components and NaN_3 as preservative (0.5 mL) 10 plastic pipettes. Secondary packaging: cardboard box.

4 - MATERIALS REQUIRED BUT NOT PROVIDED

Specimen collection container, Tubes for reaction, Disposable gloves, Timer.

5 - PRECAUTIONS AND WARNINGS

- All operations linked to the use of the test must be performed in accordance with Good Laboratory Practices.
- Crypto+Giardia Card Plus is a qualitative *in vitro* diagnostic, for professional use only; it is to be used by adequately trained and qualified laboratory personnel.
- This product is not classified as dangerous according to current European legislation.
- Avoid touching the nitrocellulose with your fingers.
- All the specimens should be considered potentially hazardous and handled in the same manner as an infectious agent.

Mascia Brunelli S.p.A., Viale Monza 272, 20128 Milan, Italy.
Tel. +39 02 25209.1, Fax +39 02 2576428
E-mail: mktg@masciabrunelli.it; export@masciabrunelli.it; web: www.masciabrunelli.it

- Each test device and each extraction buffer vial are for single use only.
- Never use reagents from another lot.
- The test should remain in the sealed pouch until use, and the test must be carried out within 2 hours of opening the sealed bag.
- Do not use the test if pouch is damaged.
- The presence of yellow lines in the results window (control and test line zone) that are visible before using the test are completely normal. That not means failure on test functionality.
- Wear gloves when handling the sample.
- Disposable gloves, extraction buffer, test tubes, and used devices in a propre biohazard container.
- The reagents' quality cannot be guaranteed beyond their shelf-life date or if the reagents are stored under inappropriate conditions.
- The Certificates of Analysis and the Safety Data Sheet of the product are available on the website www.masciabrunelli.it.
- If the device contains raw materials of animal origin. The raw material involved is derived from animals that have been slaughtered in an authorized slaughterhouse and, following an antemortem inspection, which have not shown any sign of disease transmissible to humans or animals. In any case is recommended that the kit be treated as potentially infectious, and handled observing the usual specific precautions: do not ingest, inhale, or allow to come into contact with skin, eyes, mucous membranes.
- The information provided in this document has been defined to the best of our knowledge and ability and represents a guideline for the proper use of the product but without obligation or liability. In all cases existing local laws, regulations and standard procedures must be observed for the examination of samples collected from human and animal organic districts, for environmental samples and for products intended for human or animal consumption. Our information does not relieve our customers from their responsibility for checking the suitability of our product for the intended purpose.
- Notify Mascia Brunelli Spa and the Relevant Authorities of any serious incidents occurring in connection with the in vitro diagnostic device. complaint@masciabrunelli.it

6 - Storage conditions and shelf life

Upon receipt, store the kit in their original pack at refrigerated or room temperature (2-30°C/36-89°F). If properly stored, the kit may be used up to the expiration date. The device test must remain in the sealed pouch until use. Do not use the device test after 2 hours of opening sealed-bag. Do not freeze.

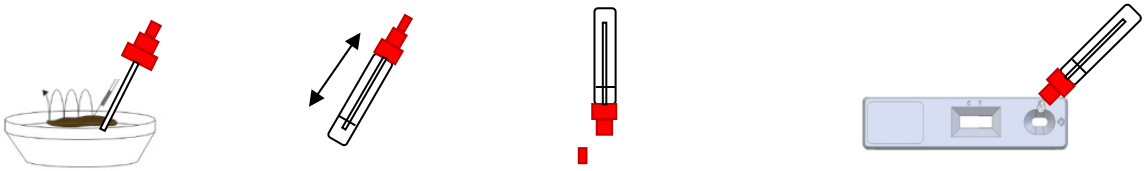
7 – Specimens and Preparation

Stool specimen should be collected as soon as possible after onset of symptoms. Collect sufficient quantity of feces (1-2 g or mL for liquid sample). Stool samples should be collected in clean and dry containers (no preservatives or transport media). The samples can be stored in the refrigerator (2-8ºC) for a maximum of **3 days** prior to testing. For longer storage, maximum **1 month**, the specimen must be kept frozen at -20ºC. In this case, the sample will be totally thawed and brought to room temperature before testing. Homogenize stool sample as thoroughly as possible prior to preparation. Freezing and thawing cycles are not recommended.

Preparation of samples

Unscrew the top of the extraction tube. Collect the stool sample with the tip of the collection device by dipping in *four* different places of the same stool specimen. Verify to transfer a small portion 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. For **Liquid or Semi-Solid Stools** use a separate pipette for each stool, draw stool of the sample itself. Dispense 125 µL of each stool into a separate extraction tube. Mix carefully and make sure the sample is well dispersed.



8 - Test procedure

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

1. Remove the test card from the protective pouch. Identify the plastic cassette with the patients data.
2. Gently shake the test tube containing the sample under investigation.
3. Brake the tip of the test tube and squeeze 3 drops of the extracted mixture into the sample well "S" of the card. Avoid to add solid particles with the liquid.
4. Read the result at 10 minutes after dispensing the sample. Do not exceeded *10* minutes.

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

9 – Control procedures

Add the requested volume (2-3 drops (about 100 µL) of Positive/Negative Controls into the sample well of the device and read the test results 10 minutes.

10 - Reading and interpretation

Interpret the results as follow:

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

		Interpretation of results
1.	- RED - BLUE + GREEN	Absence of *Cryptosporidium and Giardia.* Negative result.
2.	+ RED + BLUE + GREEN	Presence of *Cryptosporidium and Giardia.* Infection from *Cryptosporidium* and *Giardia (*α1-giardin and/or CWP1).
3.	+ RED - BLUE + GREEN	Presence of *Cryptosporidium.* Infection from *Cryptosporidium*.
4.	- RED + BLUE + GREEN	Presence of *Giardia.* Infection from *Giardia (*α1-giardin and/or CWP1).
5.	- RED - BLUE - GREEN	Invalid results, we recommend repeating the assay using the same sample with another test.

INVALID: Total absence of any control coloured line (GREEN) regardless the appearance or not of the test lines (RED or BLUE). Insufficient specimen volume, incorrect procedural techniques or deterioration of the reagents are mostly the main reasons for control lines failure. Review the procedure and repeat the assay with a new test. If the symptoms or situation still persists, discontinue using the test kit and contact your local distributor.

NOTES ON THE INTERPRETATION OF RESULTS

The intensity of the red and blue coloured bands in the test lines (T) in the results windows will vary depending on the concentration of antigens present in the specimen. However, neither the quantitative value nor the rate of increase in antigens can be determined by this qualitative test.

11 - INTERNAL QUALITY CONTROL

The Internal Quality Control procedure is included in each test strip. A line appearing in the control region (C) is an internal control. It confirms sufficient specimen volume and correct procedural technique.

12 – EXPECTED VALUES

Parasitic diseases are incriminated in causing more than 33% of global deaths of which intestinal parasitic infections are believed to take the major share. Lack of safe drinking water and environmental sanitation are largely responsible for more than 800 million expected cases of diarrheal diseases and 4.5 million associated deaths in many developing countries every year (3).

Morbidity and mortality due to diarrheal diseases in developing countries remain to be the main public health problems that need due attention. Although there could be many other causes of diarrhea, the enteric protozoa *Cryptosporidium parvum* and *Giardia lamblia* have been recognized as important causes of both out break-related and sporadic diarrhea among human beings. Both immunocompetent and immunocompromised individuals could be the victims of diarrheal diseases caused by these parasites.

13 - PERFORMANCES CHARACTERISTICS

Sensitivity and Specificity

A study was performed on faeces samples, using the Crypto+Giardia Card Plus versus Evaluation Criteria. Evaluation Criteria: two rapid test were evaluated (MB Crypto+Giardia and MB Crypto dipstick/MB Giardia Card). The discrepant results were confirmed by qPCR technique. The results were:

		Evaluation Criteria (Crypto)		
		+	**-**	**Total**
Crypto+Giardia CARD PLUS	+	33	0	33
	-	2	91	93
	Total	35	91	126

	Mean value	Confidence Interval 95% CI
Sensitivity	94.3%	80.8 – 99.3%
Specificity	100.0%	96.0 – 100.0%
PPV	100.0%	89.4 – 100.0%
NPV	97.8%	92.4 – 99.7%

		Evaluation Criteria (Giardia)		
		+	**-**	**Total**
Crypto+Giardia CARD PLUS	+	44	2	46
	-	1	79	80
	Total	45	81	126

	Mean value	Confidence Interval 95% CI
Sensitivity	97.8%	88.2 – 99.9%
Specificity	97.5%	91.4 – 99.7%
PPV	95.7%	85.2 – 99.5%
NPV	98.8%	93.2 – 100.0%

Cross reaction

An evaluation was done to determine the cross reactivity of Crypto+Giardia Card Plus. There is not cross reactivity with common gastrointestinal microorganisms and/or substances or faecal markers occasionally present in feces: *Adenovirus, Astrovirus, calprotectin; bovin, pig and human haemoglobin; human lactoferrin; human transferrin, A and B Toxins of C. difficile, C. difficile GDH, Clostridium perfringens, Entamoeba histolytica, Helycobacter pylori, Campylobacter coli e jejuni, E.ColiO157:H7, E.ColiO111; E.ColiO26; Legionella pneumophila; Listeria monocytogenes, Norovirus GI and GII; Rotavirus; Salmonella spp, Shigella spp, Streptococco pneumoniae; Streptococco pyogenes; Yersinia enterocolitica O:3 and O:9.*

14 - LIMITATIONS OF THE METHOD

- An excess of sample could cause wrong results (brown bands appear). Dilute the sample with the extraction liquid and repeat the test.
- Crypto+Giardia Card Plus should only be used on human faecal samples. The use of other samples has not been established. The quality of the test depends on the quality of the sample; proper faecal specimens must be obtained.
- Use only fresh samples or fresh frozen samples. Do not use specimens treated with solutions containing formaldehyde or its derivatives.
- A positive result determines the presence of parasites in the sample (qualitative determination) and should be used exclusively for the determination of Cryptosporidium and Giardia antigens (α-1 giardin and/or CWP1) in feces. Neither a quantitative data nor the rate of antigen increase can be determined with this test.

- 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.
- A positive result should be followed up with additional laboratory techniques (biochemical methods or microscopy) to confirm the result. 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.
- A negative result does not preclude the presence of parasites because they may be present at a concentration below the detection limit. If clinical symptoms persist, further testing by other clinical methods (e.g., microscopy) is recommended.
- Mucous and/or bloody stool samples could cause non-specific reactions in the test. Mucous and/or bloody stool samples whose result is positive should be followed up with other techniques to confirm the result.

15 - REFERENCES

1. MARSHALL, M.M., et al., "Waterborne Protozoan Pathogens", Clinical Microbiology Review, Jan. 1997, pp 67-85.
2. DYLAN R. PILLAI and KEVIN C, KAIN, "Immunochromatographic Strip-Based Detection of Entamoeba histolytica-E. dispar and Giardia lamblia Coproantigen". Journal of Clinical Microbiology, Sept. 1999, Vol. 37, No 9, p. 3017-3019.
3. LYNNE S. GARCIA et al., "Commercial Assay for Detection of Giardia lamblia and Cryptosporidium parvum Antigens in Human Fecal Specimens by Rapid Solid-Phase Qualitative Immunochromatography", Journal of Clinical Microbiology, Jan. 2003, Vol. 41, No. 1, p. 209- 212.
4. ADAM RODNEY. Biology of Giardia lamblia. Clin Microbiol Rev. 2001;14(3):447-475. doi:10.1128/CMR.14.3.447-475.2001).
5. GAÉTAN FAUBERT. Immune Response to Giardia duodenalis. Clin Microbiol Rev. 2000; 13(1): 35-54. 0893-8512/00/$04.00+.

TABLE OF APPLICABLE SYMBOLS

IVD	In Vitro Diagnostic Medical Device		Temperature limitation	LOT	Batch code (DXXX)		Manufacturer		Keep dry	UDI	Unique device identifier
	Consult Instructions for use		Use by (year/month)	REF	Catalogue number		Do not reuse		Fragile, handle with care		Keep away from heat

REVISION HISTORY

Version	Description of changes	Date
Instructions for Use (IFU) - Revision 1	Updated layout and content	2022/12

Note: minor typographical, grammatical, and formatting changes are not included in the revision history.