

Crypto/ Giardia Duo-Strip, Crypto/ Giardia Uni-Strip, Crypto/ Giardia -CIT

***In vitro* Rapid Diagnostic Test for the detection of *Cryptosporidium* and/or *Giardia lamblia* in stool specimen**

FOR IN VITRO USE

FOR PROFESSIONAL USE ONLY

Reference : C-1018, 25 tests per kit

C-1518, 10 tests individually packed, sampling devices

C-1218, 20 tests individually packed

EN

I. INTRODUCTION

The parasitic infections remain a very severe problem of health all over the world

Cryptosporidium and *Giardia* are genera of protozoan parasites that infect a wide range of vertebrates. Species within these genera cause human cryptosporidiosis and giardiasis, which probably constitute the most common causes of protozoal diarrhoea worldwide, and lead to significant morbidity and mortality in both the developing and developed world. Transmission is through the faecal-oral route following direct or indirect contact with the transmissive stages (*Cryptosporidium* oocysts and *Giardia* cysts), including person to person, zoonotic, waterborne, foodborne transmission.

Giardia lamblia (syn *Giardia duodenalis*, *G. intestinalis*) is known to be responsible for one of the main causes of persistent diarrhoea in the developed world. Trophozoites are mainly excreted during the short-lived acute phase while cysts are voided in stools during the chronic phase that follows. Typical symptoms include diarrhoea, flatulence, upper intestinal cramps, abdominal distension, nausea and weight loss.

Microscopic examination is still the method of choice to detect a giardiasis. More and more ELISA methods are now available since microscopy is time consuming and need some skill.

Cryptosporidiosis is one of the main causes of persistent diarrhoea in the developed world. It is caused by the presence of *Cryptosporidium parvum* oocysts in the gastro-intestinal tract. This parasite is known to be highly pathogenic and its infectious stage is transmitted by faecal-oral contract. It is also an opportunistic pathogen found in immunocompromised patients. The symptoms of cryptosporidiosis are watery diarrhoea, stomach cramps, weight loss, nausea, and fever. In industrialized countries 2-2.5% of diarrhoeal hospitalized patients shed *C. parvum* oocysts. Ten percent of AIDS patients have chronic cryptosporidiosis and this figure can be as high as 40% in certain developing countries *C. parvum* is diagnosed by either Ziehl-Neelsen stain or immunofluorescence in smears of unconcentrated specimens. Several ELISA tests are also available for specific detection of oocyst antigens. New molecular biology methods, such as PCR, may be used to detect these parasites in the water supply or in specimens from asymptomatic carriers. All of these methods are extremely sensitive and must be used by experienced operators. Coris BioConcept has developed a rapid diagnostic test test that can detect simultaneous the oocysts of *Cryptosporidium parvum* and the antigens of *Giardia* in unconcentrated faecal samples within 15 minutes.

II. PRINCIPLE OF THE TEST

This test is ready to use and is based on the homogeneous membrane system technology with colloidal gold particles. This device allows detection of both *Giardia lamblia* or *Cryptosporidium* in stool specimen. The specificities come from monoclonal antibodies directed against either *Giardia lamblia* or *Cryptosporidium* antigens.

This device consists of two separated sticks each specific either for the *Cryptosporidium* or for the *Giardia lamblia* and that would be placed back to back. This device shows two specific active sides (Fig #1). One side is sensitized with monoclonal antibodies specific for *Cryptosporidium* (side A) while the other side is sensitized with a monoclonal antibody specific for *Giardia lamblia* (side B). Result readings are carried out on both sides.

When the strip is dipped into the sample solution, conjugates dried in the application membranes located on both sides of the stick are solubilized and migrate along with the sample. If *Cryptosporidium* is present in the sample, a complex formed between the anti-*Cryptosporidium* conjugate and the antigen will be caught by the specific anti-*Cryptosporidium* monoclonal antibody coated on the A side of the stick. If the sample contains *Giardia lamblia*, a complex will be formed with the anti-*Giardia* conjugate and it will be caught by the *Giardia* monoclonal antibody coated on the B side of the stick. Results appear in 15 minutes in the form of a red line that develops on the strip

The solution continues to migrate to encounter a third reagent (an anti-chicken IgY polyclonal antibody) that binds the migration control conjugate, thereby producing the red control line that confirms that the test is working properly.

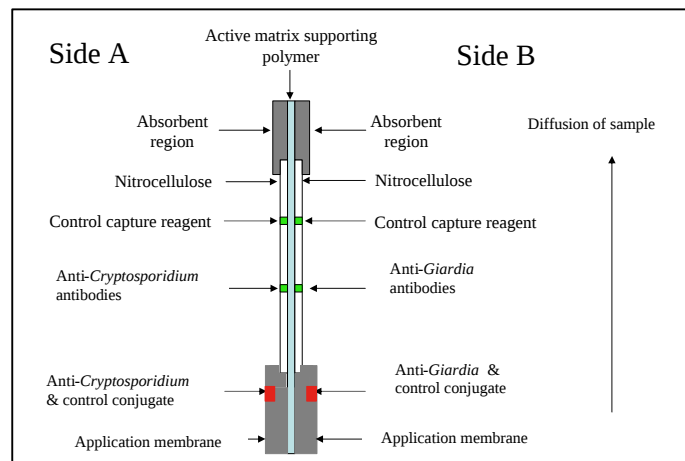


Fig #1

III. REAGENTS AND MATERIALS

Each kit contains Crypto/Giardia-Duo Strips, buffer, instruction for use and optimal components (for C-1518)

1. Crypto/ Giardia Duo-Strip

Each strip is sensitized on one side (A) with monoclonal antibodies anti-*Cryptosporidium* and with a goat anti-chicken IgY and on the other side (B) with a mouse monoclonal anti-*Giardia* and goat anti-chicken IgY.

Conjugates are made of monoclonal antibodies to either the *Cryptosporidium* or *Giardia* antigens or chicken antibody, conjugated to gold particles. These conjugates are dried on their specific sides located at the bottom of the stick in the application matrix.

These strips come in a bottle or in a pouch with a desiccant bag.

2. Dilution Buffer (15 mL)

Saline solution buffered to pH 7.5 with Tris and containing EDTA, Na₃N (<0.1%), a detergent, and blocking proteins.

3. Instruction for use (1)

4. Required materials (supplied with C-1518 catalog number)

- 3 or 5 mL test tubes
- Device for samples handling.
- cardboard rack

IV. SPECIAL PRECAUTIONS.

- All operations linked to the use of the test must be performed in accordance with the Good Laboratory Practices (G.L.P).
- The Crypto/Giardia-Strips are for *in vitro* diagnostic use only.
- Avoid touching the nitrocellulose with your fingers.
- Wear gloves when handling the samples.
- Dispose of gloves, swabs, test tubes, and sensitized strips in accordance with GLP.
- Never use reagents from another kit.
- If strips are stored in container, the container must be resealed as soon as the necessary number of strips for the operation has been removed, for the strips are sensitive to humidity. Make sure the dessicant packet is present.
- If strips are stored in individual pouches, pouch must be opened with care to avoid damaging the strip.
- Two green lines on each face indicate the immunoreagents adsorption sites. The upper lines are the migration control lines and the lower lines are the test lines. The green color disappears during the course of the test.
- Discard the dilution buffer if it is contaminated with bacteria or mould.
- The reagents' quality cannot be guaranteed beyond their expiration dates or if the reagents are stored under inappropriate conditions.
- To avoid diluting the colloidal gold conjugate in the solution, take care not to immerse the strip above the line placed under the arrows.

V – WASTE DISPOSAL

Each user is responsible for the management of any waste produced, and must ensure that it is disposed of in accordance with applicable legislation.

VI. STORAGE .

Unopened Crypto/Giardia –Duo Strip tube or pouches may be kept at between 4 and 30°C and used until the expiration date indicated on the packaging.

The strips remain stable for 15 weeks (**in the closed container**) after the tube is opened if they are kept between 4 and 30°C and in a dry environment.

Crypto/Giardia-Strips and buffer must not be frozen.

Real-time long-term stability is under evaluation. Intermediate results are available at CORIS BioConcept.

VII. SAMPLES.

The stool specimens must be tested as soon after they are collected as possible. If necessary, they may be stored at 2-8°C for 24 hours or -20°C for longer periods of time. Make sure that the specimens are not treated with solutions containing formaldehyde or its derivatives.

VIII. PROCEDURE .

PREPARATIONS :

If the Crypto/Giardia Duo-Strip kit was kept at 4°C, let all the reagents in the unopened packaging warm up to room temperature before proceeding with the test.

Indicate the specimen number on the test tube (prepare one test tube per sample).

Place the marked test tubes in a rack.

SPECIMEN PREPARATION PROCEDURE:

1. Add 0.5 ml or 15 drops of the dilution buffer solution to each tube.
2. Dip the inoculating loop containing the stool sample into the tube. The dilution ratio must be at most 4% w/v.
 - For liquid samples, take 2 loops of 10 µL,
 - For solid samples, take 1 loop.
3. Stir to homogenize the solution and let stand for 1-2 minutes.
4. Discard the inoculating loop and immerse the sensitized strip in the direction indicated by the blue arrow.
5. Let react 15 minutes.

Results must be read on wet strips after 15 minutes incubation.

IX. INTERPRETING THE RESULTS

The side with the orange sticker is specific for the *Cryptosporidium*

The side with the pink sticker is specific for the *Giardia*

Results are to be interpreted as follows, for each side:

- 1 line (upper) = negative
- 2 lines = positive
- 0 line = invalid*

*The absence of the migration control line for each side, which is the upper line, makes the result invalid. In this case, the sample must be retested.

The intensity of the test lines may vary according to the quantity of antigens found in the sample. Any signal, even weak, on the test line must be regarded as a positive result. Nevertheless, the test is qualitative and cannot predict the quantity of antigens present in the sample. The clinical presentation and other test results must be taken into consideration to establish diagnosis.

During the drying process, a very faint shadow may appear at the test line. It should not be regarded as a positive result.

To store the results, let the strip dry after removing the absorbent material at its base.

X. PERFORMANCE.

A. Detectability :

Detection limit has been realised with a quantified solution of *Giardia* cysts and has been evaluated at 3125k/mL.

B. Sensitivity- Specificity (Correlation) :

An evaluation has been performed to characterize the kit and has been conducted on 100 stool samples in comparison with an Immuno Enzymatic rapid Test.

Following results have been obtained:

CRYPTOSPORIDIUM

Immuno enzymatic Test	Positive	Negative	Total
Crypto (/Giardia) strip			
Positive	45	0	45
Negative	2	53	55
Total	47	53	100

Sensitivity : 95.7 % (45/47)

Positive Predictive Value: 100% (45/45)

Specificity : 100 % (53/53)

Negative Predictive Value: 96.4% (53/55)

	Consult instructions for use		Do not reuse		REF		Manufactured by
	Keep dry		Use by	IVD	In vitro diagnostic medical device		Temperature limitation
DIL AS	Diluent assay	CONT NaN3	Contains Sodium azide		Contains sufficient for <n> tests	DIL SPE	Diluent specimen

GIARDIA

Immuno enzymatic Test	Positive	Negative	Total
(Crypto) /Giardia strip			
Positive	43	0	43
Negative	5	52	57
Total	48	52	100

Sensitivity : 89.6 % (43/48)

Positive Predictive Value : 100% (43/43)

Specificity : 100% (52/52)

Negative Predictive Value: 91.2 % (52/57)

C. Accuracy

To check the intra-lot accuracy, one *giardia* positive sample and one *Cryptosporidium* positive sample, and a buffer solution (as negative control sample) have been tested 15 times on sticks of the same production lot in the same experimental conditions. All observed results were similar as expected.

To check the inter-lot accuracy, same samples (positive in *Giardia* and in *Cryptosporidium* and dilution buffer) were tested on three different production lots. All results were similar as expected.

D. Interference

Cross-reactivity to samples positive for the following pathogens was tested and found to be negative: *Salmonella typhimurium*, Coronavirus, *Entamoeba histolytica*, *Entamoeba dispar*, several *E. coli* strains (including *E. coli* O157:H7 and *E. coli* c600-933W), *Rotavirus*, *Adenovirus*, *Cryptosporidium parvum* (for side *Giardia*), *E.coli* F5, *Salmonella enteritidis*, *Giardia lamblia* (for side *Crypto*), *Giardia muris*.

Tests for cross-reactivity has been tested on *Staphylococcus aureus* and found positive at high bacteria concentrations

XI. LIMITS OF THE KIT

Crypto/Giardia Duo-Strip kit results must be compared with all other available clinical and laboratory information.

A positive test does not rule out the possibility that other pathogens may be present.

The Crypto/Giardia Duo-Strip is an acute-phase screening test. Stool specimens that are collected after this phase may contain antigen titres below the reagent's sensitivity threshold. If a sample is given a negative result despite the observed symptoms, a culture should be started to check the sample

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Manufacturer :

CORIS BIOCONCEPT

Science Park – CREALYS

Rue Jean Sonet 4A

B – 5032 GEMBLOUX

BELGIUM

Tel : +32(0)81.719.917

Fax : +32(0)81.719.919

E-mail : info@corisbio.com

<http://www.corisbio.com>

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