INSTRUCTIONS FOR USE

CAMPYLOBACTER SPECIES AG CARD PLUS

RAPID IMMUNOCHROMATOGRAPHIC TEST FOR THE QUALITATIVE DETECTION OF *CAMPYLOBACTER SPP* (IDENTIFIES THE PATHOGENIC SPECIES *CAMPYLOBACTER JEJUNI* AND *CAMPYLOBACTER COLI*) IN HUMAN FECES AND IN SUSPECTED COLONIES, INCLUDING CONTROLS

1 – INTRODUCTION AND INTENDED USE

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

Campylobacteriosis is the disease caused by the presence of *Campylobacter spp*. The common routes of transmission are faecal-oral, person to-person sexual contact, ingestion of contaminated food or water, and the eating of raw meat. The onset of disease symptoms usually occurs two to five days after infection, but can range from one to ten days.

There are 16 species and 6 subspecies assigned to the genus *Campylobacter*, of which the most frequently reported in human disease are *C. jejuni* (subspecies jejuni) and *C. coli* (99% C. jejuni). *C. laridis* and *C. upsaliensis* are also regarded as primary pathogens, but are generally reported far less frequently in cases of human disease.

The most common clinical symptoms of *Campylobacter* infections include diarrhoea (frequently with blood in the faeces), abdominal pain, fever, headache, nausea, and/or vomiting. The symptoms typically last three to six days.

Campylobacter Species Ag Card Plus is a manual, rapid immunochromatographic test for the qualitative detection of *Campylobacter spp.* (identifies the pathogenic species *Campylobacter jejuni* and *Campylobacter coli)* in feces specimens and *Campylobacter* suspected colonies in stool culture. The test offers a simple and highly sensitive screening assay to make a presumptive diagnosis of *Campylobacter* infection (campylobacteriosis) and it could be used to identify of suspected isolates of *Campylobacter* from selective media (stool culture).

2 - PRINCIPLE OF THE METHOD

Campylobacter Species Ag Card Plus is an non-invasive, simple to perform, rapid and very accurate immunochromatographic method for the determination of *Campylobacter* in stool samples and *Campylobacter* suspected colonies in stool culture.

The strip consists of a nitrocellulose membrane pre-coated with mouse monoclonal antibodies on the test line (T), in the results window, against *Campylobacter* 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-campylobacter) 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 positive for *Campylobacter,* the antigen of the diluted sample reacts with the red-coloured conjugate complex (anti-campylobacter 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-campylobacter antibodies present on the membrane (test line) capture the coloured conjugate and the red line will be visible. This band is used to interpret the result.

If the sample is negative, there is no *Campylobacter* 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 red-coloured conjugate complex. The anti-campylobacter antibodies present on the membrane (test line) will not capture the antigen-red-coloured conjugate complex (not formed), for which the red line will not appear. Whether the sample is positive or not, the mixture continues to move across the membrane to the immobilized specific antibodies placed in the control line. The anti-specific protein antibodies present on the membrane will capture control green-conjugate complex and the control line will always appears. The presence of this green line serves 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
Campylobacter Species Ag Card Plus CND: W0105011401; EDMA: 14.70.01.90; RDM: 1424106/R	Immunochromatographic test	**VC1007P** (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 as negative control. (25 x 1 mL). 1 glass dropper bottle containing Positive Control: mixture with non infectious components and NaN_3 as preservative (1 x 0.5 mL) Secondary packaging: cardboard box.

4 - MATERIALS REQUIRED BUT NOT PROVIDED

Specimen collection container, Tubes for test, Plastic droppers, 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.
- Campylobacter Species Ag 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.
- 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.

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- 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.

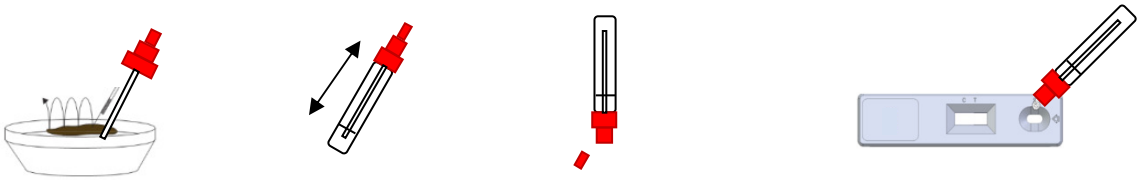
7 – Specimens and Preparation

Faecal samples: Stool samples should be collected in clean and dry containers. The samples can be stored in the refrigerator (2-8ºC) for **1-2 days** prior to testing. For longer storage, maximum **1 year**, 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.

Samples preparation

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. 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 samples**, add approx. 125µL in the stool collection tube using a micropipette. Close the tube with the diluent and stool sample. Shake the tube in order to assure good sample dispersion.



Suspected Campylobacter colonies in stool culture. Use selective media for the isolation of Campylobacter (microaerobic atmosphere, 48 hours/42°C). After 48 hours of incubation in selective media the typical Campylobacter colonies will be growth.

1. Examine the plates after 2 days incubation. Select Campylobacter typical colonies. Take out the cap of the collection tube. Use the stick or an inoculating needle to pick up 3 or 4 suspected Campylobacter colonies and add them to the collection tube.
2. Close the tube with the diluent and suspected colonies. Shake the tube in order to assure good sample dispersion.

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. Brake the tip of the test tube.
3. Squeeze 3 drops of the extracted mixture into the sample well "S" of the card. Avoid adding solid particles with the liquid.
4. Read the result at 10 minutes after dispensing the sample. Do not exceeded *10* minutes.

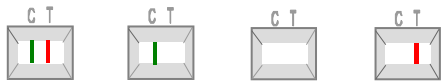
If the test does not run due to solid particles, stir the sample added in the sample window (S) with the stick. If it doesn´t work, dispense a drop of diluent until seeing the liquid running through the reaction zone.

9 – Procedure for Controls

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

10 – Reading and Interpretation

Interpret the results as follow:



Positive Negative Invalid

	Campylobacter	Interpretation of results
1.	-	There is no *Campylobacter* presence. No infection caused by *Campylobacter*. Negative result.
	GREEN	
2.	+	There is *Campylobacter* presence. *Campylobacter* infection, which might mean diarrhoea, abdominal pain, fever, headache, nausea and/or vomiting.
	RED-GREEN	
3.	OTHER RESULTS	Invalid result, 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 line (RED). 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 coloured band in the test line (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

Campylobacter spp are bacteria that are a major cause of diarrhoeal illness in humans and are generally regarded as the most common bacterial cause of gastroenteritis worldwide. In developed and developing countries, they cause more cases of diarrhoea than, for example, foodborne *Salmonella* bacteria. In developing countries, *Campylobacter* infections in children under the age of two years are especially frequent, sometimes resulting in death. In almost all developed countries, the incidence of human *Campylobacter* infections has been steadily increasing for several years. The reasons for this are unknown.

13 - PERFORMANCES CHARACTERISTICS

Analytical sensitivity (detection limit)

Detection limit values for the different species are:

For *Campylobacter jejuni* detection: The lower detection limit value is: 3.12ng/mL of *Campylobacter jejuni* recombinant protein.
The typical detection limit value is: 0.78ng/mL of *Campylobacter jejuni* recombinant protein.

For *Campylobacter coli* detection: The lower detection limit value is: 3.12ng/mL of *Campylobacter coli* recombinant protein.
The typical detection limit value is: 0.78ng/mL of *Campylobacter coli* recombinant protein.

Clinical sensitivity and specificity

It was performed an evaluation using Campylobacter Species Ag Card Plus vs a commercial qPCR kit (VIASURE Campylobacter Real Time PCR Detection Kit). The 113 specimens were obtained from patients with the same as *Campylobacter* infection symptoms.
Campylobacter Species Ag Card Plus showed 93,7% of sensitivity, 98% of specificity, PPV 98,3% and NPV 92,5%.

Cross reaction and Interferences

It was performed an evaluation to determine the cross reactivity of Campylobacter Species Ag Card Plus. There is not cross reactivity with common intestinal pathogens, other organisms and substances occasionally present in faeces: *Adenovirus, Astrovirus, C. difficile antigen GDH, Clostridium perfringens, Cryptosporidium, Entamoeba dispar/histolytica, E. coli O:111, O149, O157:H7, Giardia, H. pylori, Legionella, Listeria monocytogenes, Norovirus GI/GII, Rotavirus, Salmonella, Shigella, Staphylococcus aureus, Streptococcus pneumoniae, Streptococcus pyogenes, Yersinia enterocolitica O3/O9.*

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.
- Campylobacter Species Ag 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.
- Freezing and thawing cycles for the sample are not recommended, it could cause wrong results.
- A positive result determines the presence of *Campylobacter* in the sample (qualitative determination) and can be due to a variety of causes and/or species. Neither a quantitative figure nor the rate of antigen increase can be determined with this test.
- A positive result must be followed by further laboratory techniques to confirm the results. However, confirmation of infection should only be made by the physician after evaluation of all clinical and laboratory findings and should be based on correlation of the results with further clinical observations.
- A negative result is not meaningful because of it is possible the antigens concentration in the stool sample is lower than the detection limit value. If the symptoms or situation still persist, a *Campylobacter* determination should be carried out on a sample from an enrichment culture.
- 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. Fernández, H. and Farace, M.I. "Manual de Procedimientos Campylobacter". INEI. 2003.
2. Kawatsu, K. et al. "Development and Evaluation of Immunochromatographic Assay for Simple and Rapid Detection of Campylobacter jejuni and Campylobacter coli in Human Stool Specimens". Journal of Clinical Microbiology Apr. 2008 Vol 46, No. 4, p. 1226-1231.

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 4	Updated layout and content; alignment to the italian version revision index	2023/01

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

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