# RAPID TESTS FOR
# INFECTIOUS DISEASES

Respiratory
Parasitology
Gastroenterology
Sexually Transmitted Diseases


• Easy • Rapid • Accurate
• Room temperature storage
• CE Mark • Complete Kit



Mascia Brunelli

# RAPID TESTS

| INFECTIOUS AGENT | PRODUCT NAME | DETECTION | SPECIMEN | FORMAT | CODE | TESTS |
|---|---|---|---|---|---|---|
| | **GASTROENTEROLOGY** | | | | | |
| H.pylori | **HELICOBACTER PYLORI CARD Ab** | IgG Abs | WB, S, P | Card | VQ81650 | 20 tests |
| | **HEPY STOOL CARD PLUS** | Antigen | Stool | Card | VT82001P | 50 tests |
| C.difficile | **CLOSTRIDIUM DIFFICILE TOXIN A+B CARD PLUS** | Antigen | Stool | Card | VC194055P | 25 tests |
| | **CLOSTRIDIUM DIFFICILE GDH** | Antigen | Stool | Card | VC194065 | 25 tests |
| Campylobacter | **CAMPYLOBACTER SPECIES Ag CARD** | Antigen | Stool | Card | VC1007 | 25 tests |
| E.coli O157 | **O157 E.COLI CARD** | Antigen | Stool | Card | VC1010 | 25 tests |
| | **E.COLI VEROTOXINS 1&2** | Antigen | Stool | Card | VC1031 | 10 tests |
| Salmonella | **SALMONELLA Ag** | Antigen | Stool | Card | VQ84060 | 10 tests |
| Adenovirus | **ADENOVIRUS CARD PLUS** | Antigen | Stool | Card | VC194020P | 25 tests |
| Rotavirus | **ROTAVIRUS CARD PLUS** | Antigen | Stool | Card | VC194022P | 25 tests |
| Adeno+Rota | **ADENO+ROTA CARD PLUS** | Antigen | Stool | Card | VC194025P | 25 tests |
| Astrovirus | **ASTROVIRUS -DIPSTICK** | Antigen | Stool | Dipstick | VC1020 | 25 tests |
| Norovirus | **NOROVIRUS** | Antigen | Stool | Card | VC1027 | 25 tests |
| Hepatitis A Virus | **HAV IgM CARD** | IgM Abs | S, P | Card | VR82005 | 30 tests |
| | **RESPIRATORY** | | | | | |
| Legionella | **LEGIONELLA PNEUMOPHILA CARD PLUS** | Antigen | Urine | Card | VQ84100P | 25 tests |
| Adenovirus | **ADENO-RESPI-CARD PLUS** | Antigen | NP swab | Card | VC1014P | 25 tests |
| Enterovirus | **ENTEROVIRUS** | Antigen | Stool | Card | VC1026 | 25 tests |
| RSV+Adenovirus | **RSV+ADENO-RESPI DIPSTICK** | Antigen | NP swab | Dipstick | VC1019 | 25 tests |
| RSV | **RSV-RESPI CARD PLUS** | Antigen | NP swab | Card | VC1015P | 25 tests |
| Influenza | **INFLU A+B RESPI** | Antigen | NP swab | Dipstick | VC1012 | 25 tests |
| Strep A | **STREP A CARD PLUS** | Antigen | Throat swab | Card | VQ81210P | 50 tests |
| M.tubercolosis | **TUBERCOLOSIS** | IgM-IgG Abs | WB, S, P | Card | VQ81800 | 10 tests |
| | **SEXUALLY TRANSMITTED DISEASES** | | | | | |
| C.trachomatis | **CHLAMYDIA TRACHOMATIS CE 0483** | Antigen | V, U swab | Card | VQ81406 | 20 tests |
| Gardnerella | **GARDNERELLA VAGINALIS** | Antigen | V swab | Dipstick | VQ81601 | 20 tests |
| Trichomonas | **TRICHOMONAS VAGINALIS** | Antigen | V swab | Dipstick | VQ81604 | 20 tests |
| N.gonorrheae | **GONORREA Ag CARD** | Antigen | V, U swab | Card | VQ81602 | 25 tests |
| Syphilis | **SYPHILIS Ab** | Total ABS | WB, S, P | Card | VQ83000 | 25 tests |
| Strep B | **STREP B CARD** | Antigen | V swab | Card | VQ81310 | 50 tests |
| | **PARASITOLOGY** | | | | | |
| Cryptosporidium | **CRYPTO DIPSTICK** | Antigen | Stool | Dipstick | VC1005 | 25 tests |
| Giardia | **GIARDIA CARD** | Antigen | Stool | Card | VC1016 | 25 tests |
| Crypto+Giardia | **CRYPTO+GIARDIA CARD** | Antigen | Stool | Card | VC1023 | 25 tests |
| Crypto/Giardia/Entam | **CRYPTO/GIARDIA/ENTAMEBA** | Antigen | Stool | Card | VC1032 | 10 tests |
| Entameba | **ENTAMEBA** | Antigen | Stool | Card | VC1030 | 10 tests |
| Malaria | **MALARIA MBPan** | Antigen | WB | Card | VQ81706 | 30 tests |
| Leishmania | **LEISHMANIA CARD** | IgM-IgG Abs | WB, S, P | Card | VQ85200 | 10 tests |
| Filaria | **FILARIASIS CARD** | IgM-IgG Abs | WB, S, P | Card | VQ85300 | 25 tests |
| | **OTHERS** | | | | | |
| Leptospira | **LEPTOSPIRA CARD** | IgM-IgG Abs | WB, S, P | Card | VQ85100 | 10 tests |
| Dengue | **DENGUE Ag NS1 - IgG/IgM** | Ag-IgM-IgG | WB, S, P | Card | VQ84006 | 25 tests |

**KEY:** WB Whole Blood - S Serum - P Plasma - NP Nasopharingeal - V Vaginal - U Urethral - EC Endocervical

Products “PLUS” (code ending “P”) contain the Controls

CE IVD

Mascia Brunelli

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# STREP A CARD

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

**Immunochromatographic test for detection of Group A Streptococcus from throat swabs**

## I. INTENDED USE

Strep A Card is a lateral flow, immunoassay for the rapid, qualitative detection of Group A Streptococcal antigen from throat swabs. Beta-haemolytic Group A Streptococcus is a major cause of upper respiratory infection such as tonsillitis, pharyngitis and scarlet fever in kids.

## II. PRINCIPLE

The Strep A Card utilizes two site sandwich immunoassay technology for the detection of Group A Streptococcal antigen. The method utilizes a combination of specific antibody conjugate with colloidal gold on solid surface. During testing, the Strep A antigen is extracted from the throat swab using Extraction Reagents 1 and 2. The extracted solution is then added to the sample well. The Strep A antigen reacts with colored antibody-colloidal gold conjugate to form Strep A antigen-antibody complexes. The mixture then moves chromatographically across the membrane to the immobilized rabbit anti-Strep A antibody at the test line region. If Strep A antigen is present in the specimen, a red colored sandwich of solid phase/Strep A antigen/gold conjugate is formed on the test line region (T). Absence of the red line at the test line region indicates a negative result. Regardless of the presence of Strep A antigen, as the extracted mixture continues to move laterally across the membrane to the immobilized goat anti-rabbit antibody test region, a red line at the control region will always appear (C). The presence of this colored band serves as: 1) verification that sufficient volume has been added, 2) verification that proper flow is obtained and 3) reagent control.

## III. REAGENTS AND MATERIALS

Each kit contain:
1) **Strep-A Cards**: sealed pouch containing the device, an essicant and a tube for sample extraction, with dropper tip
2) **Extraction solution 1**
3) **Extraction solution 2**
4) **Sterile Swabs**
5) **Extraction tube**
6) **Instructions leaflet**

## IV. STORAGE AND STABILITY

The test kit is to be stored at temperature (4-30°C) in the sealed pouch for the duration of the shelf-life.

## V. PRECAUTIONS

1) For in vitro diagnostic use only.
2) For professional use only.
3) Read the package insert instruction before use the kit.
4) Do not use beyond the expiration date which appears on the package label.
5) As with all diagnostic tests, a definitive clinical diagnosis should not be based on the results of a single test, but should only be made by the physician after all clinical and laboratory findings have been evaluated.

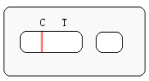
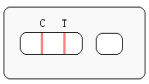
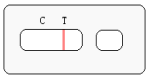
## VI. SPECIMEN COLLECTION

To obtain optimal results use only swabs in rayon or dracon. Do not use calcium alginate, cotton tipped swabs.
It is recommended that swabs specimens be processed as soon as possible after collection. If swabs are not processed immediately, they should be placed into a sterile, dry, tightly capped tube or bottle and refrigerated. Swabs can be stored at room temperature up to 4 hours, or refrigerated (4-8°C) up to 5 days.

## VII. TEST PROCEDURE

1) Remove the test card from the sealed pouch.
2) Place the specimen swab in the plastic tube supplied. Add 6 drops of Extraction Reagent 1 (300 µl) and 6 drops of Extraction Reagent 2 (300 µl). Swirl vigorously to mix the reagents. Then incubate the mixture at room temperature for 2-5 minutes.
3) After this time, expunge as much liquid as possible from the swab by pressing and rotating the fiber portion against the wall of the tube. Discard the swab.
4) Put the dropper tip on to the extraction tube. Holding the sample dropper above the card, squeeze a total of 4 drops (200 µl) of the mixed specimen into the sample well (S).
5) Interpret test results at 10 minutes. Do not interpret test after 10 minutes.

## VIII. INTERPRETATION OF RESULTS

| | | |
|---|---|---|
| Negative: | The control line appears in the test window, but the test line is not visible. |  |
| Positive: | Two colored lines should be observed in the viewing region. The line in the test region **(T)** is the probe line; the line in the control region **(C)** is the control line, which is used to indicate proper performance of the test. |  |
| Invalid: | No line appears in the control region. Under no circumstances should a positive sample be identified until the control line forms in the viewing area. If the control line does not form, the test result is inconclusive and the assay should be repeated. |   |

## IX. PERFORMANCE CHARACTERISTICS

**Analitical Sensitivity:** To determine the analytical sensitivity of the Strep A Card, Group A Streptococcus bacteria were grown in broth culture. The detection limit of the Rapid Strep A Test was determined to be $2.5 \times 10^5$ organisms per test. Sensitivity: **$2.5x10^5$ org/test.**

**Specificity:** To determine the specificity of the Strep A Card to Group A Streptococcal bacteria, the following Group A Streptococcal Strains at different levels of organisms per test were examined. Positive results obtained at the level of 2.5 × $10^5$ organisms/test for all Strep A strains indicate that Strep A Card is sensitive to all Group A Streptococcal bacteria. Cross-reactivity studies with organisms likely to be found in the respiratory tract were also performed using the Strep A Card. The following organisms were tested at 1 × $10^8$ organisms/test.

| **ORGANISMS** | **RESULT** |
|---|---|
| Strep-A (2.5x$10^5$ org/test) strains: SS-091; SS-410; SS-492; SS-496; SS-633; SS-634; SS-635; SS721; SS-754; SS-799; ATCC-19615 | + |
| Strep-B, -C, -D, -F, -G | - |
| Pseudomonas aeruginosa | - |
| Streptococcus bovis, faecalis, mitis, mutans, salivarius, pneumoniae, sanguis | - |
| Staphylococcus aureus, epidermidis, saprophyticus | - |
| Proteus vulgaris | - |
| Escherichia coli | - |
| Corynebacterium diphtheriae | - |
| Neisseria lactima, gonorrhoeae, meningitidis, sicca, subflava | - |
| Bordetella pertussis | - |
| Moraxella catarrhalis | - |
| Candida albicans | - |
| Haemophilus parahaemophyticus | - |

**Accuracy**
A correlation study of the Strep A Card and the conventional culture tests has been determined in multi-center clinical evaluations. Throat swab specimens were taken from patients exhibiting symptoms of pharyngitis.
The qualitative results are summarized as follows:

| | | Culture | | |
|---|---|---|---|---|
| | | Positive | Negative | Total |
| Strep A Card | Positive | 21 | 3 | 24 |
| | Negative | 2 | 35 | 37 |
| | Total | 23 | 38 | 61 |

Sensitivity: 21/23 = 91.3% Specificity: 35/38 = 92.1% Accuracy: (21+35)/61 = 91.8%

## X. EXTERNAL QUALITY CONTROL
Positive and negative control are available in Catalogue Mascia Brunelli ( UD80025).

## XI. LIMITATION OF PROCEDURE
The accuracy of the test dependents on the quality of the swab sample. False negative may result from improper sample collection or storage. A negative result may be obtained from patients at the onset of the disease due to low antigen concentration. Therefore, when a patient suspected of having infection, additional testing using the culture method is required.
The test does not differentiate asymptomatic carriers of Group A Streptococcus from those with infection.
Respiratory infections, including pharyngitis, can be caused by Streptococci from serogroups other than Group A, as well as by other pathogens.
As for any diagnostic procedure, the results obtained with this test should be used in conjunction with other information available to the physician. Strep A Card is a test for the qualitative detection of Group A Streptococcal antigen.

## XII. REFERENCES
1. Facklam RR and Carey RB "Streptococci and Aerococci, Manual of Clinical Microbiology" 4th ed, Lenette EH, Balows A, Hausler WJ and Shadomy H, *American Society for Microbiology*, 1985.
2. Levinson ML and Frank PK "Differentiation of Group A from other Beta Hemolytic Streptococci with Bacitracin" *J, Bacteriol* 69,284-287 (1995).
3. Edwards EA, Phillips IA and Suiter WC "Diagnosis of Group A Streptococcal Infections Directly from Throat Secretions" *J. Clin. Micro.*, 15,481-483 (1982).
4. Gupta R, Talwar GP and Gupta SK "Rapid Antibody Capture Assay for Detection of Group A Streptococci Using Monoclonal Antibody and Colloidal Gold-Monospecific Polyvalent Antibody Conjugate" *J. Immunoassay*, 1992, 13, 441-445.
5. Rose PW "Throat Swabs and Swabbing Technique" *The Practioner*, 207,791-796 (1971).
6. Lauer BA, Rellar LB and Mirrett S "Effect of Atmosphere and Duration of Incubation on Primary Isolation of Group A Streptococci from Throat Cultures" *J.Clin. Micro* 17,338-340 (1983)

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| IVD | 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 |

| **CONTENTS** | **REF. VQ81210 (50 test)** | **REF. VQ81209 (20 test)** |
|---|---|---|
| 1) Strep-A Cards | 50 items | 20 items |
| 2) Extraction solution 1 | 1 x 16,5 mL | 1 x 7 mL |
| 3) Extraction solution 2 | 1 x 16,5 mL | 1 x 7 mL |
| 4) Sterile Swabs | 50 items | 20 items |
| 5) Instructions leaflet | 1 item | 1 item |

**EDMA Code 15 70 01 03 00** CE IVD

# INFLU-A+B-RESPI-DIPSTICK

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

**Immunochromatographic test strip for the detection of Respiratory Influenza A and B Viruses in nasopharyngeal secretions (swabs, washing or aspirates)**

## I. INTRODUCTION AND INTENDED USE

Influenza is a highly contagious viral infection of the upper respiratory tract that is characterized by the antigen variability, the seasonality and the impact on the general population.
Of the two main (A and B) types of influenza viruses, Influenza A subtypes are differentiated by antigens variability of the surface glycoproteins (haemagglutinin, H and neuraminidase, N). Influenza A virus is the most prevalent and is associated with the most serious epidemics. Influenza A has 3 subtypes which are important for humans: A(H3N2), A(H1N2) and A(H1N1), of which the former is currently associated with most deaths.
Influenza can cause severe complications such as bronchitis or pneumonia, particularly in children, elderly people or those with chronic respiratory disease. It is most often a mild viral infection transmitted by respiratory secretions through sneezing or coughing. There are many other viral infections that can mimic influenza, making laboratory tests necessary to distinguish it from other acute respiratory infections.
Virus isolation is still considered as the gold standard method for the Influenza diagnosis, with a sensitivity of nearly 100% after 3 days. Patients health care and economic costs can be greatly improved by the use of rapid, specific and sensitive antigen detection method in order to allow the use new antiviral treatments.
Influ-A+B-Respi-Dipstick is a non-invasive lateral flow assay for the detection of Respiratory Influenza A and B viruses in nasopharyngeal secretions.

## II. PRINCIPLE

This is a ready-to-use qualitative immunochromatographic test based on lateral flow principle for detection of Influenza A and B viruses.The membrane is pre-coated with monoclonal antibodies against *Influenza A and B* antigens on the test line region. During testing, the sample reacts with the particle coated with anti-Influenza antibodies which were pre-dried on the test strip. The mixture moves upward on the membrane by capillary action. In the case of a positive result the specific antibodies present on the membrane will react with the mixture conjugate and generate coloured lines (one (A/B) or two (A or B) 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 contain:
**1. Influ-A+B-Respi-Dipstick (25 test):** tube containing 25 reactive strips and dissecant.
**2. Dilution buffer (1 x 12,5 mL):** saline solution buffered to pH 7,5, containing $NaN_3$ (< 0.1%), a detergent and charged proteins..
**3. Instruction for use (1)**

**Required materials (not supplied)**

Specimen collection container, Disposable gloves, Plastic pipettes, testing tubes or vials, Timer or clock.

## IV. PRECAUTIONS

- All operations linked to the use of the test must be performed in accordance with Good Laboratory Practices.
- The kit is for in vitro diagnosis only.
- Avoid touching the nitrocellulose with your fingers
- Wear gloves when handling the samples.
- Disposable gloves, swabs, test tubes, and sensitized strips in accordance with GLP
- Never use reagents from another lot.
- The tube containing the sensitized strips must be recapped as soon as the necessary number of strips for the operation has been removed, since the strips are sensitive to humidity. Make sure that the desiccant is present.
- Discard the dilution buffer if it is contaminated with bacteria or mould.
- The reagents' quality cannot be guaranteed beyond their shelf-life date or if the reagents are stored under inappropriate conditions.
- As with all diagnostic tests, a definitive clinical diagnosis should not be based on the results of a single test, but should only be made by the physician after all clinical and laboratory findings have been evaluated.

## V. STORAGE

Store as packaged in the sealed pouch either at refrigerated or room temperature (2-30ºC). 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. SAMPLES AND PREPARATION

Specimens to be tested should be obtained and handled by standard methods. The use of transport media has not been validated on the kit.

**NASOPHARYNGEAL SWAB METHOD:**

- Bend shaft to follow curve of nasopharynx. Insert swab through nostril to posterior nasopharynx.
- Rotate swab a few times to obtain infected cells.
- For an optimal sample, repeat procedure using other nostril.

**NASOPHARYNGEAL ASPIRATE METHOD (SUCTION APPARATUS, STERILE SUCTION CATHETER):**

- Instill several drops of solution saline into each nostril.
- Place catheter through nostril to posterior nasopharynx. Apply gentle suction. Using rotating motion, slowly withdraw catheter.
- For an optimal sample, repeat procedure using other nostril.

Send specimen to lab immediately (testing sensitivity decrease over time). Cool specimen to 2º-4ºC (36º-40ºF) during storage and transport.

## VII. PROCEDURE

**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.**

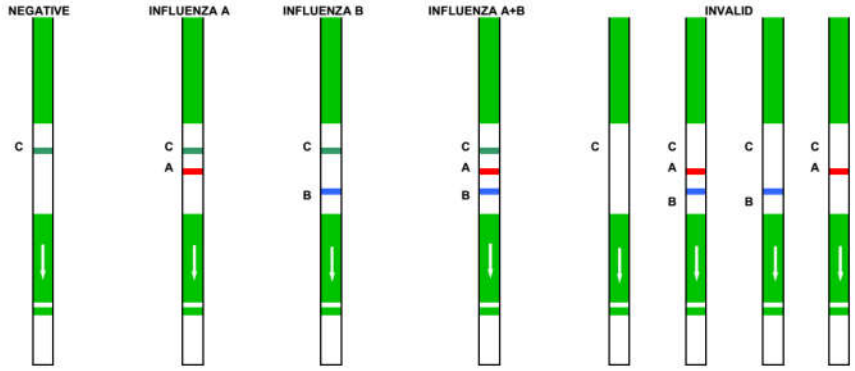
**To process the collected nasopharyngeal wash or aspirate samples:**

Use a separate pipette and testing tube for each sample. Add the nasopharyngeal wash or aspirate sample (6 drops or 300uL) in a testing tube or vial. Add the diluent Buffer (3 drops or 150uL) and mix. Extract some of the liquid and dispense 150uL in a new testing tube. Remove the Influenza A+B Strip from its sealed pack and use it as soon as possible. Leave the test strip to stand vertically taking care of not surpassing the limit of immersion indicated with the arrows. Start the timer. Read the result at 10 minutes after dispensing the sample.

**To process the collected nasopharyngeal swab :**
Use a separate testing tube or vial for each sample (swab). Add the diluent Buffer (10 drops or 500uL) into the testing tube or vial, put the nasopharyngeal swab, mix and extract as much liquid possible from the swab. Extract some of the liquid and dispense 150uL in a new testing tube. Remove the *Influenza A+B* Strip from its sealed pack and use it as soon as possible. Leave the test strip to stand vertically taking care of not surpassing the limit of immersion indicated with the arrows. Start the timer. Read the result at 10 minutes
To avoid diluting the latex conjugate in the solution, take care not to immerse the strip above the line placed under the arrows.

## VIII. INTERPRETING THE RESULTS

**Negative test:** In the case only one line appears in the control line region (green control line).
**Positive test:** In the case two or three lines appears across the central window, in the Result Line Region (red/blue test line) and in the control line region (green control line).
**Invalid test:** The absence of the migration control line, which is the upper line, makes the result invalid. In this case, the sample must be retested.




## IX. INTERNAL QUALITY CONTROL

Internal procedural controls are included in the test. A GREEN line appearing in the control region (C) is an internal control. It confirms sufficient specimen volume and correct procedural technique.

## X. PERFORMANCE CHARACTERISTICS

**A. Sensitivity – Specificity - Correlation**
Different virus extract preparation:
Influenza A/New Caledonia/20/99 (**H1N1) strain** (15 µg/mL hemagglutinin) Influenza A/ (**H2N2) strain** (15 µg/mL hemagglutinin)
Influenza A/Fujian/411/2002 (**H3N2) strain** (15 µg/mL hemagglutinin) Influenza A/ (**H7N7) strain** (15 µg/mL hemagglutinin)
Influenza B/Shanghai/**361/2002 strain (**15 µg/mL hemagglutinin)) Influenza A/ (**H9N2) strain** (15 µg/mL hemagglutinin)
was diluted in the sample diluent and tested (with 4 different lots) in accordance with the kit instructions for use.
We found that, under such conditions, the detection limit using the reference antigen preparation of Influenza A and B is **4.7 ng/mL HA for Influenza A and 18.75 ng/mL HA for Influenza B.**
The **correlation** has been conducted on 115 NPS swab samples in comparison with commercial rapid test ( Quidel and Binax Now Influenza A&B) shows a 99% **Sensitivity** and 99 % **Specificity for** both Influ Aand B
An evaluation compared with *RT-PCR technique *resulted:
Sensitivity **(novel H1N1)** 67% Sensitivity **(seasonal flu)** 84%
**B. Reproducibility:** To check the intra-lot accuracy, one Influenza A positive sample and one Influenza B positive sample, and a dilution buffer solution (as negative control sample) have been tested 10 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 Influenza A and in Influenza B and dilution buffer) were tested on three differents production lots. All results were similar as expected.
**C. Interference:** Cross-reactivity to samples positive for the following pathogens was tested and found to be negative: Adenovirus, HSV, Parainfluenza, Enterovirus, Rhinovirus, Nocardia asteroides, Respiratory syncytial virus, Streptococcus pneumoniae, Moraxella catarrhalis, Streptococcus pyogenes, Aspergillus niger, Legionella pneumophila, Candida albicans, Haemophilus influenzae.

## XI. LIMITS OF THE TEST

- The test must be carried out within 2 hours of opening the sealed pack.
- Samples containing blood or erythrocytes should be avoided for testing since they can lead to false positive.
- A positive result does not rule the possibility that other pathogens may be present.
- Kit results must be compared with all other available clinical and laboratory information.
- The kit is an acute-phase screening test. NPS 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.

## XII. BIBLIOGRAPHIC REFERENCES

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2. Cox, N. J. and Subbarao, K. (2000). "Global epidemiology of influenza: past and present." Annu Rev Med 51: 407-21
3. Hayden, F. G. and Palese, P. (2002). Influenza Virus. pp. 891-920, in: Clinical Virology. D. D. Richman, R. J. Whitley and F. G. Hayden, eds. Washington, ASM Press
4. I. Renuart, P. Mertens and T. Leclipteux. 2002. "An immunochromatographic test for the detection of Influenza-A Virus." European Biotech Crossroads, October 2002, Lille, France
5. M. Lynch. "Prospective evaluation of an optical immunoassay for detection of Influenza during the 1999-2000 seasons." Clinical Virology Laboratory, Fairview-University Medical Center, University of Minnesota, Minneapolis, MN
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| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| IVD | 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)** **Ref. VC1012**
Influ-A+B-Respi-Dipstick 25 items
Diluent buffer 1 x 12,5 mL
Instruction for use 1 item

**EDMA (EDMS) CODE 15709090** CE IVD

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INSTRUCTIONS FOR USE

# CAMPYLOBACTER SPECIES AG CARD

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

## 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 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 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<br>CND: W0105011401;<br>EDMA: 14.70.01.90;<br>RDM: 1424105/R | Immunochromatographic test | **VC1007**<br>(25 tests) | 25 sealed in foil pouch containing the device, with dessicant.<br>25 plastic tubes with dropper tip containing the extraction liquid. To use also as negative control. (25 x 1 mL).<br>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 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.
- 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.

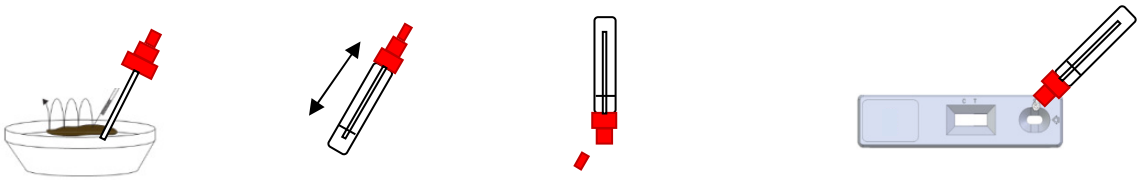
## 7 – SPECIMENS AND PREPARATION

**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

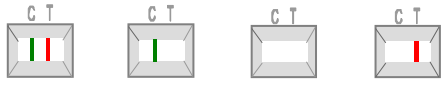
**Allow the tests, stool samples and buffer to reach to room temperature (15-30ºC/59-86ºF) prior to testing. Do not open pouches until ready to perform the assay.**

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 – 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.

## 10 - 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.

## 11 – 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.

## 12 - 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 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 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. 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.*

## 13 - 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 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.

## 14 - 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 3 | Updated layout and content | 2023/01 |

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

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# SALMONELLA Ag

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

**Immunochromatographic rapid test for the qualitative detection of *Salmonella spp* in faeces and in food samples**

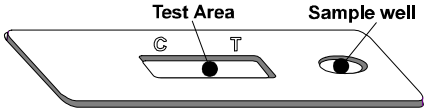
## I. INTRODUCTION AND INTENDED USE

The *Salmonella* Ag is chromatographic immunoassay for the qualitative detection of *Salmonella spp.* in stool samples in order to detect salmonellosis in persons and in contaminated food samples in order to avoid consuming it and salmonellosis disease.
Clinical syndromes in humans caused by infection with *Salmonella enterica* are divided into typhoid fever, caused by *S. enterica serovars typhi* and *paratyphi*, and a range of clinical syndromes, including diarrhoeal disease, caused by the non-typhoid *salmonellae* (NTS) of which there are around 2,500 serovars. Typhoid fever is a human-restricted and highly adapted invasive systemic disease of adults and children that shows little association with immunosuppression. In contrast, NTS have a broad vertebrate host range and epidemiology that often involves food animals, at least in industrialised countries where it usually presents as gastroenteritis. Severe, invasive disease due to NTS is usually associated with the immunocompromised state common in HIV-infected adults. Invasive NTS disease is also common in young African children with co-morbidities such as severe anaemia, malnutrition and HIV infection.
*Salmonella* Ag provides a rapid detection of *Salmonella spp.* directly from the faecal samples and directly from enrichment food samples (meat, dairy).

## II. PRINCIPLE OF THE TEST

The Salmonella Ag is a qualitative immunoassay for the detection of *Salmonella* in food samples and in faecal samples. The membrane is pre-coated with antibodies, on the test band region, to recognize Salmonella spp antigen. During testing, the sample is allowed to react with the coloured latex particles coated with anti-*salmonella* antibodies which were pre-dried on the test. The mixture then moves upward on the membrane by capillary action. As the sample flows through the test membrane, the coloured particles conjugate migrate. In the case of a positive result the specific antibodies present on the membrane will capture the coloured particles (conjugate). The mixture continues to move across the membrane to the immobilized antibody placed in the control band region, a GREEN coloured band always appears. 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:

1. Salmonella Ag (10 card)
2. Extraction buffer (8 mL x 1Vial)
3. Instruction for use (1)

### Required materials (not supplied)

Testing tubes, specimen collection container, disposable gloves and container, plastic pipette and timer.
*Salmonella* Enrichment media: Rappaport-Vassiliadis (RVS broth) and pre-enrichment media: Peptone Buffered Water. Stomacher and Stomacher bags, Incubators +37ºC and +41.5ºC. and Purified water.

## IV. SPECIAL PRECAUTIONS

- For professional *in vitro* diagnostic use only.
- Do not use after expiration date.
- The test should remain in the sealed pouch until use.
- Do not use the test if pouch is damaged.
- Follow Good Laboratory Practices, wear protective clothing, use disposal gloves, do not eat, drink or smoke in the area.
- All the specimens should be considered potentially hazardous and handled in the same manner as an infectious agent.
- The test should be discarded in a proper biohazard container after testing.
- The test must be carried out within 2 hours of opening the sealed bag.

## 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 FOR STOOL SAMPLES

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 or transport media). The samples can be stored in the refrigerator (2-4ºC/36-40ºF) for 1-2 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.

## VII. PROCEDURE FOR STOOL SAMPLES

**To process the collected stool samples**

Use a separate swab or stick, dropper and testing tube or vial for each sample. Dispense 0.7 mL (or 14 drops) of extraction buffer into a testing tube. Collect the stool sample with the tip of the collection device by dipping in two different places of the same stool specimen. Verify to transfer a small portion (150 mg) of stool. Put the collection device back into the testing tube. Shake the extraction tube in order to get an homogeneous solution. For liquid or semi-solid stools using a separate pipette, draw stool of the sample itself. Dispense 150 µL of each stool into a testing tube with extraction tube (dispense 1.0 mL (or 20 drops). Mix carefully, then vortex 15 seconds.

**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 card from its sealed pouch and use it as soon as possible.
2. Use a separate device for each sample. Extract some liquid from the topside with a dropper.
3. Dispense 4 drops or 100µL into the specimen well. Start the timer.
4. Read the result at **10 minutes** after dispensing the sample.

## VIII. SPECIMENS COLLECTION FOR FOOD SAMPLES

Food samples should be collected in clean containers and the assay should be done right after collection. The samples can be stored in the refrigerator (2-4 ºC) for 1-2 days prior to testing. For longer storage, the specimen must be kept frozen at –20ºC. In this case, the sample will be totally thawed, and brought to room temperature before testing. Ensure only the amount needed is thawed because of freezing and defrosting cycles are not recommended. Homogenise sample as thoroughly as possible prior to preparation.

**Sample enrichment:**
- Mix 25 g of solid sample or 25 mL of liquid sample with 225 mL of Buffered Peptone Water (REF 5112783); if necessary, homogenize with a homogenizer of peristaltic type Bagmixer 1 (REF 7221230) .
- Incubate for 18 ± 2 h at 37°C ± 1°C.
- Transfer 1 mL of the culture obtained to a tube containing 10 mL of the Rappaport-Vassiliadis RVS broth (REF 551981).
- Incubate the inoculated RVS Broth at 41,5°C ± 1°C for 24 h ± 3 h

## IX. PROCEDURE FOR FOOD SAMPLES

**Allow the devices, samples and controls to reach to room temperature (15-30ºC) prior to testing. Do not open pouches until ready to perform the assay.**

1. Place 1.0-2.0 mL (approximately 30-40 drops) of enrichment culture in a testing tube and cover it.
2. Place tubes in boling water bath for 15 minutes. Remove and allow to reach to room temperature.
3. Remove the card from its sealed bag just before using and identify it.
4. Use a separate pipette and device for each sample or control. Dispense 4 drops or 100 µL into the circular window marked with an arrow, avoiding to add solid particles with the liquid.
5. Read the result at 10 minutes (the coloured bands appear).

## X. INTERPRETING THE RESULTS

**NEGATIVE:** Only one GREEN control band appears across the central window in the site marked with the letter C (control line).

**POSITIVE:** In addition to the GREEN control band across the central window in the site marked with the letter C (control line), a RED band (test line) also appears in the site marked with the letter T (result region).

**INVALID**: A total absence of the control coloured band. Insufficient specimen volume, incorrect procedural techniques or deterioration of the reagents are likely the reasons for control line failure. Review the procedure and repeat the tests using a new test.


**POSITIVO** **NEGATIVO** **NON VALIDO**

## XI. INTERNAL QUALITY CONTROL

Internal procedural controls are included in the test. A GREEN line appearing in the control region (C) is an internal control. It confirms sufficient specimen volume and correct procedural technique.

## XII. PERFORMANCE

### A. Expected Values

Typhoid fever and salmonellosis are public health problems in developing countries, where the incidence of cases per year is 200–500/100 000. Transmission occurs by contamination of water or food with bacteria. Animals and humans are the principal reservoirs.

### B. Detection Limit

The detection limit for the different serotypes is: *S. enteritidis* $1x10^4$bacteria/mL, *S. typhimurium* $1x10^4$bacteria/mL and *S. typhi*: $1x10^7$bacteria/mL.

### C. Sensitivity and Specificity

It was performed an evaluation using Salmonella Ag (Mascia Brunelli). It was studied 40 stool samples and the results were confirmed by Singlepath®Salmonella. Salmonella Ag (Mascia Brunelli) showed >99% of sensitivity and >97% of specificity.
The antibodies used to elaborate this test recognise *Salmonella* epitopes found in stool of patients, as well as in preparations from the bacteria cultures in vitro. This preliminary values has to be taken with precaution until more evaluation data will be available.

### D. Cross-Reactivity and interferences

It was performed an evaluation to determine the cross reactivity of Salmonella Ag. There is not cross reactivity with common intestinal pathogens, other organisms and substances occasionally present in faeces: *H. pylori, Escherichia coli O157:H7, Listeria monocytogenes, Campylobacter.*

## XIII. LIMITS OF THE KIT

- The test must be carried out within 2 hours of opening the sealed bag.
- An excess of sample could cause wrong results (brown bands appear). Dilute the sample with the buffer and repeat the test.
- Some stool samples can decrease the intensity of the control green line.
- Freezing and thawing cycles for the sample are not recommended, it could cause wrong results.
- A negative result is not meaningful because it is possible the *Salmonella* content in the stool sample to be too small. A *Salmonella* determination should be carried out on a sample from a enrichment culture.
- This test provides a presumptive diagnosis of salmonellosis in faece or absence or presence of Salmonella in food sample. A confirmed infection diagnosis or positive result should only be made by a physician after all clinical and laboratory findings have been evaluated must be based in the correlation of the results with further clinical observations.

## XIV. REFERENCES

- GORDON, M, et al, "Invasive salmonellosis in Malawi". J Infect Developing Countries 2008; 2(6):438-442.
- SANCHEZ-JIMENEZ, M. et al. "Validation of a PCR for diagnosis of typhoid fever and salmonellosis by amplification of the hilA gene in clinical simples from Colombian patients", Journal of Medical Microbiology (2004), 53, 875–878.

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| IVD | 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 (10 tests)** **Ref. VQ84060**

| | | |
|---|---|---|
| SALMONELLA Ag | | 10 Device (Card) |
| Extraction Buffer | | 1 vial x 8 mL |
| Instruction for use | 1 item | |

***EDMA Code 15 01 10 01 00*** CE IVD

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**INSTRUCTIONS FOR USE**

# O157 E. COLI CARD

**RAPID IMMUNOCHROMATOGRAPHIC TEST FOR THE QUALITATIVE DETECTION OF *O157-E. COLI* IN HUMAN FAECES, FOOD SAMPLES AND IN SUSPECTED COLONIES**

## 1 – INTRODUCTION AND INTENDED USE

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

Infection with *Escherichia coli O157:H7* (Enterohemorrhagic Escherichia coli, EHEC) presents with a wide spectrum of clinical manifestations, including asymptomatic carriage, nonbloody diarrhoea, haemorrhagic colitis, the haemolytic-uremic syndrome (HUS), and thrombotic thrombocytopenic purpura (TTP). Not only is *Escherichia coli O157:H7* an important agent for haemorrhagic colitis, it is also one of the leading causes of bacterial diarrhoea.
Transmission of *Escherichia coli O157:H7* is primarily food-borne. Undercooked meat is the most common culprit, dairy products and secondary person-to-person spread are also important. The organism produces at least two Shiga-like toxins. These toxins are thought to have direct pathogenic significance in *Escherichia coli O157:H7* infection. This infection is usually diagnosed from a positive stool culture, from the presence of Shiga toxins, or both. Timely collection (within 7 days of illness onset) of a stool sample for culture is imperative for a high recovery rate.
O157 E. COLI CARD is a manual, rapid immunochromatographic test for the qualitative detection of *Escherichia coli O157:H7* in food and in human stool samples, to aid in the diagnosis of *E. coli* infections. It is possible to detect the *E. coli O157:H7* from suspected colonies in stool culture. The test offers a simple and highly sensitive screening assay to make a presumptive diagnosis of *Escherichia coli O157:H7* infection and it could be used to identify of suspected isolates of *E. coli O157:H7* from selective media.

## 2 - PRINCIPLE OF THE METHOD

O157 E. COLI CARD is an non-invasive, simple to perform, rapid and very accurate immunochromatographic method for the determination of *Escherichia coli O157:H7* in food specimens, in stool samples and *E. coli O157:H7* suspected colonies in stool culture.
The strip consists of a nitrocellulose membrane pre-coated with antibodies on the test line (T), in the results window, against *Escherichia coli O157:H7* 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 (antibodies anti-*Escherichia coli O157:H7*) 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 *Escherichia coli O157:H7,* the antigens of the diluted sample react with the red-coloured conjugate complex (anti-*Escherichia coli O157:H7* 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-*Escherichia coli O157:H7* 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 *Escherichia coli O157:H7* antigen presence and yet, the antigens 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-*Escherichia coli O157:H7* 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 |
|---|---|---|---|
| O157 E. COLI CARD<br>CND: W0105011501;<br>EDMA: 15.01.15.01;<br>RDM: 1465288/R | Immunochromatographic test | **VC1010**<br>(25 tests) | 25 sealed in foil pouch containing the device, with dessicant.<br>1 plastic bottle with dropper tip containing the extraction liquid. (1 x 20mL).<br>5 plastic pipettes.<br>Secondary packaging: cardboard box. |

## 4 - MATERIALS REQUIRED BUT NOT PROVIDED

Specimen collection container, Tubes for test, Disposable gloves, Timer. Incubators +37°C ± 1°C and Purified water.
O157 E.COLI Enrichment media: ECBroth (Ref. Biolife 551425), Bagmixer 1 (Ref. Biolife 7221230), Sorbitol MacConkey Agar (Biolife Ref. 541669S).

## 5 - PRECAUTIONS AND WARNINGS

- All operations linked to the use of the test must be performed in accordance with Good Laboratory Practices.
- O157 E. COLI CARD 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 is 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 proper 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.

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- 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 Collection and Procedure for Stool Samples

**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**

Use a separate swab or stick, dropper and testing tube or vial for each sample. Dispense 0,7 mL (or 14 drops) of the extraction buffer into a testing tube. Collect the stool sample with a stick by dipping in *four* different places of the same stool specimen. Verify to transfer a small portion of stool (approx. 125 mg). Put the stick 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 or semi-solid samples,** use a transfer pipette, taking a quantity of faeces from the sample itself. Dispense 125µL of the faecal sample into a tube containing the extraction liquid (0.7 mL or 14 drops). Close the tube containing the diluent and stool sample. Shake the tube in order to assure good sample dispersion.

**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. Use a separate device for each sample. Extract some liquid from the topside with a dropper.
3. Dispense 4 drops into the specimen well. Start the timer.
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.

## 8 - Specimens Collection and Procedure for Food Samples

Food samples should be collected in clean containers and the assay should be done right after collection. The samples can be stored in the refrigerator (2-4 ºC) for 1-2 days prior to testing. For longer storage, the specimen must be kept frozen at –20ºC. In this case, the sample will be totally thawed, and brought to room temperature before testing. Ensure only the amount needed is thawed because of freezing and defrosting cycles are not recommended. Homogenise sample as thoroughly as possible prior to preparation.

**Sample enrichment:**

- Mix 25 g of solid sample or 25 mL of liquid sample with 225 mL enrichment medium Enrichment media: ECBroth ; if necessary, homogenize with a homogenizer for 2 min. (Bagmixer 1) .
- Incubate for 18-24 hours at 37°C ± 1°C.

**Test Procedure**

**Allow the tests, 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. Place 1 or 2 mL of enrichment samples in a testing tube and cover it. Only bring to room temperature the number of tests required to assay before opening it.
2. Use a separate device for each sample. Extract some liquid from the topside with a dropper and dispense 150 µL into the specimen wells. Start the timer.
3. Read the result at 5 minutes after dispensing the sample.

## 9 - Procedure for Plate Culture

**Suspected *E. coli O157:H7* colonies in stool culture.** The Sorbitol MacConkey Agar (Biolife Ref. 541669S) is the method of choice for the isolation of *E.coli O157:H7* (aerobic atmosphere, 24 hours/37°C). After 24 hours of incubation in selective media the typical *E. coli O157:H7* colonies will be colourless.

1. Examine the plates after 1 day incubation. Select *E. coli O157:H7* typical colonies. Dispense 0,7 mL (or 14 drops) of extraction buffer in a collection tube. Use an inoculating needle to pick up 3 or 4 suspected *E. coli O157:H7* colonies and add them to the collection tube.
2. Close the tube with the extraction buffer and suspected colonies. Shake the tube in order to assure good sample dispersion.

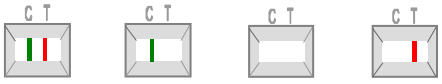
**Test Procedure**

**Allow the tests 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. Use a separate device for each sample. Extract some liquid from the topside with a dropper.
3. Dispense 4 drops into the specimen well. Start the timer.
4. Read the result at 10 minutes after dispensing the sample. Do not exceeded *10* minutes.

## 10 – Reading and Interpretation

Interpret the results as follow:




Positive Negative Invalid

| | E. coli O157:H7 | Interpretation of results |
|---|---|---|
| 1. | - | There is no *E. coli O157:H7* presence. No infection caused by *E. coli O157:H7*. Negative result. |
| | GREEN | |
| 2. | + | There is *E. coli O157:H7* presence. *E. coli O157:H7* infection, presents with a wide spectrum of clinical manifestation, including asymptomatic carriage, nonbloddy diarrhoea, haemorrhagic colitis, the haemolytic-uremic syndrome (HUS) and thrombocytopenic purpura (TTP). |
| | 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 the test. A line appearing in the control region (C) is an internal control. It confirms sufficient specimen volume and correct procedural technique.

### 12 – EXPECTED VALUES

*Escherichia coli O157:H7* causes 73,000 illnesses in the United States annually. That means 8.598 cases, 17% require hospitalizations, 4% haemolytic uremic syndrome cases and 0,5% deaths. The main transmissions routes are: 52% foodborne, 21% unknown, 14% person to person, 31% waterborne, 3% animal contact and 0,3% laboratory related.

### 13 - PERFORMANCES CHARACTERISTICS

**Analytical sensitivity (detection limit)**

Detection limit value of O157 E.COLI CARD is $1.87x10^4$ CFU/mL.

**Clinical sensitivity and specificity**

O157 E.COLI CARD was evaluated to determine sensibility in selective enrichment culture and samples, specificity with producers organisms of Shiga toxins, non-Shiga toxins producers and other Enterobacteriaceae species (Reference Laboratory for Escherichia coli – LREC).
14 STEC strains (O157:H7 antigen); 4 Non STEC strains (O157); 9 STEC strains (non O157); 4 other *Enterobacteriaceae spp*.
The results show: >99% of sensitivity, 85% of specificity, PPV 70% and NPV >99%.

**Cross reaction and Interferences**

It was performed an evaluation to determine the cross reactivity of O157 E.COLI CARD. There is not cross reactivity against gastrointestinal pathogens occasionally present in faeces: *Campylobacter coli, Campylobacter jejuni; Citrobacter freundii; C. difficile; E. coli O22:H8, O91:H-, O103:H2, O111:H21, O145:H-, O171:H2, O174:H8; Klebsiella pneumoniae; H. pylori; Listeria monocytogenes; Morganelle morganii; Proteus mirabilis; Salmonella enteritidis, paratyphi, typhi, typhimurium; Shigella boydii, dysenteriae, flexneri, sonnei; Staphylococcus aureus; Yersinia enterocolitica.*

### 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.
- O157 E.COLI CARD 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.
- A positive result determines the presence of *E. coli O157:H7* in the sample (qualitative determination); nevertheless, a positive result should be followed up with additional laboratory techniques (biochemical method or by PCR) to confirm the results. A confirmed 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, an *E. coli O157:H7* determination should be carried out on a sample from an enrichment culture.

### 15 - REFERENCES

1. THOMPSON, J., HODGE, D. and BORCZYK, A.; "Rapid Biochemical Test to Identify Verocytotoxin-Positive Strains of Escherichia coli Serotype O157"; Journal of Clinical Microbiology, Oct. 1990, Vol. 28, No. 10, pp 2165-2168.
2. VALLANCE B.A. and FINLAY B.B., "Explitation of host cells by enteropathogenic Escherichia coli", PNAS, August 2000, Vol. 97, No. 16, pp. 8799-8806.
3. BLANCO, M. et al. "Escherichia coli Verotoxigénicos (ECVT) en Espana: ECVT O157:H7 y NO-O157 en humanos y alimentos. El Ganado bovino y ovino como reservorio. Técnicas para detección de ECVT" Laboratorio de Referencia de Escherichia coli (LREC).

**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 5 | Updated layout and content | 2023/03 |

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

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