INSTRUCTIONS FOR USE

STREP A CARD

IMMUNOCHROMATOGRAPHIC TEST FOR THE QUALITATIVE DETECTION OF GROUP A *STREPTOCOCCUS* FROM THROAT SWABS AND SUSPECTED GROUP A *STREPTOCOCCAL* COLONIES RECOVERED FROM CULTURE

1 – CLINICAL SIGNIFICATE AND INTENDED USE

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

Strep A Card is a manual, lateral flow immunoassay for the rapid, qualitative detection of Group A *Streptococcal* antigens from human throat swab specimens and from suspected Group A *Streptococcal* colonies recovered from culture. The test is an aid for the presumptive diagnosis of beta-haemolytic Group A *streptococcus* infection.

Group A *Streptococcal* infections are caused by Group A *Streptococcus*, a bacterium responsible for a variety of health problems. These infections can range from mild skin infection or sore throat to severe, life-threatening conditions such as toxic shock syndrome (multi-organ failures) and necrotizing fasciitis (soft tissue disease), commonly know as flesh eating disease. Most people are familiar with strep throat, which along with minor skin infection, is the most common form of the disease. Health experts estimate that more than 10 million mild infections (throat and skin) like these occur every year.

Conventional identification procedures for Group A *Streptococcus* from throat swabs involve the isolation and subsequent identification of viable pathogens by techniques that require 24 to 48 hours or longer. STREP A CARD detects either viable or nonviable organism directly from a throat swab, providing results within 5 minutes.

2 - PRINCIPLE OF THE METHOD

The STREP A CARD is a qualitative lateral flow immunoassay for the detection of Group A *Streptococcus* antigen in human throat swab or/and suspected Group A *Streptococcal* colonies recovered from culture. The membrane is pre-coated with mouse monoclonal antibodies against Group A *Streptococcus* on the test line region and with rabbit polyclonal antibodies against a specific protein on the control line region. The label/sample adsorbent pad is sprayed with test label solution (mouse monoclonal antibodies anti-Group A *Streptococcus)* conjugated to red polystyrene latex and control label solution (specific binding protein) conjugated to blue polystyrene latex, forming coloured conjugate complexes. The *Strep A* antigen is extracted from the throat swab/colonies using Extraction Reagents 1 and 2. The extracted solution is then added to the sample well. If the sample is positive, the antigens of diluted sample react with the red-coloured conjugate complex (anti-*Strep A* monoclonal antibodies-red polystyrene microspheres) which was previously pre-dried on the adsorbent pad. The mixture moves upward on the membrane by capillary action. As the sample flows through the test membrane, the binding conjugate complexes migrate. The anti-*Strep A* antibodies present on the membrane (test line) capture the coloured conjugate and the red line will be visible. If the sample is negative, there is no Group A *Streptococcus* antigens 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-*Strep A* 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 blue-conjugate complex and the control line will always appears. The presence of this blue 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
Strep A Card CND: W0105090103 EDMA: 15.70.01.03; RDM: 1476041/R	Immunochromatographic test	**VQ81209** (20 tests)	20 sealed in foil pouch containing the device, with dessicant. 1 plastic bottle with dropper tip containing the Extraction Solution 1 (2M Sodium nitrite) – 1 x 7 mL 1 plastic bottle with dropper tip containing the Extraction Solution 2 (0,15M Acetic Acid) – 1 x 7 mL 20 sterile swabs 20 plastic droppers 20 plastic tubes Secondary packaging: cardboard box.
Strep A Card CND: W0105090103 EDMA: 15.70.01.03; RDM: 1476039/R	Immunochromatographic test	**VQ81210** (50 tests)	50 sealed in foil pouch containing the device, with dessicant. 1 plastic bottle with dropper tip containing the Extraction Solution 1 (2M Sodium nitrite) – 1 x 16,5 mL 1 plastic bottle with dropper tip containing the Extraction Solution 2 (0,15M Acetic Acid) – 1 x 16,5 mL 50 sterile swabs 50 plastic droppers 50 plastic tubes Secondary packaging: cardboard box.

Extraction Solution 1

Attention
(Sodium Nitrite)
H302; H319; H413
P264; P270; P273; P280; P330; P301+P312; P305+P351+P338; P337+P313; P501

4 - MATERIALS REQUIRED BUT NOT PROVIDED

Specimen collection container, Disposable gloves, Timer, Blood Agar Sheep (Biolife REF. 541151).

5 - PRECAUTIONS AND WARNINGS

- All operations linked to the use of the test must be performed in accordance with Good Laboratory Practices.
- Strep A 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 classified as dangerous according to current European legislation (Extraction Solution 1); (view above table and consulting the MSDS).
- 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.
- The test must be carried out within 2 hours of opening the sealed bag.
- Do not use the test if pouch is damaged.
- Wear gloves when handling the sample.
- Disposable gloves, extraction solutions, 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.
- 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.

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

Samples should be process as soon as possible after collection. If this is not possible, the samples can be stored in the refrigerator (2-8°C) for 8 hours prior to testing.

Throat swab method:

Collect the throat swab sample with a sterile swab, from the posterior pharynx, tonsils and other inflamed areas. Avoid touching the tongue, cheeks and teeth with the swab.

Process the swab as soon as possible after collecting the specimen.

Suspected Group A Streptococcus colonies:

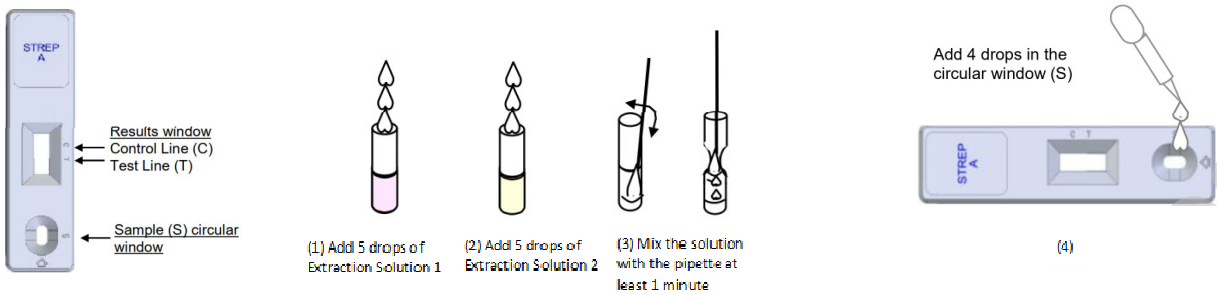
The Blood Agar is the method of choice for the isolation of Group A Streptococcus (anaerobic atmosphere, 72 hours/37°C). After 72 hours of incubation in blood the typical Group A Streptococcus colonies will be translucent white showing beta haemolysis.

1. Examine Blood plates after incubation period. Select Streptococcus typical colonies. Use a sterile swab to pick up 1 or 3 colonies suspected (showing characteristic beta haemolysis).
2. Follow the instructions in the test procedure section to test the swab.

8 - TEST PROCEDURE

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

1. Add 5 drops (1) of Extraction Solution 1 (light red) and 5 drops (2) of Extraction Solution 2 in a testing tub (the solution should turn light yellow (colourless)) immediately put the swab into the tube.
2. Mix the solution by rotating the swab forcefully against the side of the tube at least 1 minute. Best results are obtained when the specimen is vigorously extracted in the solution (3). Extract as much liquid as possible from the swab, squeezing the sides of the tube or rotating the swab against the side of the tube as the swab is withdrawn. Discard the swab.
3. Remove the test card from the sealed pouch just before using it.
4. Use a separate pipette and test for each sample. Dispense exactly 4 drops from the testing tube, into the circular window marked with the letter S (4).
5. **Read the results at 10 minutes.** Do not interpret test after 10 minutes.

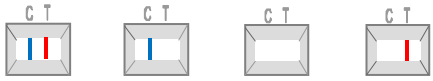


9 - READING AND INTERPRETATION

Interpret the results as follow:

NEGATIVE: In the reading window only 1 blue band appears in the control region "C". This is the control line assuring the correctness of test performing.

POSITIVE: two lines, one blue (C) and one red (T) are visible in the control and test areas of the window. The intensity of the band colour in the test region is proportionally to the antigen concentration in the sample. However, neither the quantitative value nor the rate of increase in antigens can be determined by this qualitative test.
INVALID: No band appears in the control region. A sample should never be identified as positive if you do not generate a control line. If the control line is not formed, the test is invalid and must be repeated.



Positive Negative Invalid

10 - Internal quality control

Internal procedural control is included in the test. A blue line appearing in the control line (C) in the results window is an internal control, which confirms sufficient specimen volume and correct procedural technique. The colour of the liquid changes from light pink to light yellow (colourless) as you adds Extraction Solution 2 to Solution A. This is an internal extraction reagent control. The colour change means that you mixed the extraction reagent control, that you mixed the extraction reagent properly and that the reagents are functioning properly.

11 – External Quality Control

Positive and negative control are available in Catalogue Mascia Brunelli (REF. UD80025).

12 – Expected values

Although "strep throat" can occur at any age and at any time of the year, it mainly affects school-age children during the winter and spring.

13 - Performances characteristics

Detection limit: To determine the detection limit of the STREP A CARD, different Strep A antigen preparations were diluted in a diluent and tested in accordance with the kit instructions for use. The detection limit of the STREP A CARD was determined to be 3.9×10^4 CFU/mL.

Clinical sensitivity and specificity: an evaluation, with throat specimens from patients with pharyngitis symptoms, was performed comparing the results obtained by Strep A Card test and another rapid test commercial available (OSOM® Strep A Test, Genzyme Diagnostics). The results showed a high sensitivity and specificity, both >99%.

Cross-reactivity: an evaluation was performed to determine the cross reactivity of Strep A Card; no cross reactivity against organisms that cause other respiratory infections:

Influenza type A *Influenza type B* *Adenovirus* Group D *Streptococcus: Enterococcus*

14 - Limitations of the method

- This test does not differentiate between carriers and acute infection. Pharyngitis may be caused by organisms other than Group A *Streptococcus.*
- Strep A Card should be used only with throat swabs or colonies taken directly from a plate. The use of swab specimens taken from other sites or the use of other samples such as saliva, sputum or urine has not been established. The quality of the test depends on the quality of the sample; proper throat swab specimens must be obtained.
- The intensity of test line may vary from very strong at high antigens concentration to faint when the antigens concentration is close to the detection limit value of the test.
- Positive results determine the presence of Group A *Streptococcus* infection. A confirmed infection should only be made by a physician after all clinical and laboratory findings have been evaluated and must be based in the correlation of the results with further clinical observations.
- A negative result is not meaningful because of it is possible the antigens concentration in the throat samples is lower than the detection limit value. If the symptoms or situation still persist, a Group A *Streptococcus* determination should be carried out, on a sample from an enrichment culture.
- The components of this I.v.D. were always tested together without compatibility with components from other manufacturers. While not excluding the possibility that these components can be used with components of the same formulation but produced by other companies, there is no experimental evidence of such compatibility.

15 - References

1. Vincent MT, Celestin N, Hussain AN. Pharyngitis. Am Fam Physician 2004;69:1465-70.
2. McIsaac WJ, Goel V, To T, Low DE. The validity of a sore throat score in family practice. CMAJ 2000;163:811-5.

TABLE OF APPLICABLE SYMBOLS

IVD	In Vitro Diagnostic Medical Device		Temperature limitation	LOT	Batch code (EXXX)		Manufacturer		Keep dry
	Consult Instructions for use		Use by (year/month)	REF	Catalogue number		Keep away from heat		Fragile, handle with care

REVISION HISTORY

Version	Description of changes	Date
Instructions for Use (IFU) - Revision 9	Updated layout and content	2022/10

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

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