INSTRUCTIONS FOR USE

STREP B CARD

IMMUNOCHROMATOGRAPHIC TEST FOR THE QUALITATIVE DETECTION OF GROUP B *STREPTOCOCCUS* ANTIGEN IN VAGINAL SWABS OR CULTURE

1 – CLINICAL SIGNIFICATE AND INTENDED USE

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

Strep B Card is a manual, lateral flow immunoassay for the rapid, qualitative, presumptive detection of Group B *Streptococcus* antigens from human vaginal swab specimens and from suspected Group B *Streptococcus* colonies recovered from culture. The test is an aid for the presumptive diagnosis of Strep B infection.

Group B Streptococci (GBS) or Streptococcus agalactiae are among the most frequent causes of life-threatening infectious in neonates. Between 5% and 30% of all pregnant women are colonized with GBS.[1] Several recent studies have shown that the intrapartum treatment of GBS-colonized women significantly reduces the incidence of GBS-caused sepsis.[2-4] The US Center for Disease Control and Prevention (CDC) recommends routine examination for Group B streptococcus between the 35th and the 37th week of pregnancy. A CDC study has shown that routine examinations is 50% more effective than the use of antibiotics for pregnant women with clinical risk factors. Standard culture methods require 24 to 48 hours, and the results may not be available soon enough for efficient treatment. Thus, methods utilizing more rapid screening techniques are required.

2 - PRINCIPLE OF THE METHOD

The Strep B Card test detects Group B Streptococcus antigens through visual interpretation of color development on the internal strip. Anti-Strep B antibodies are immobilized on the test region of the membrane. During testing, the specimen reacts with polyclonal anti-Strep B antibodies conjugated to colored particles and precoated onto the sample pad of the test. The mixture then migrates through the membrane by capillary action, and interacts with reagents on the membrane. If there is sufficient Strep B antigen in the specimen, a colored band will form at the test region of the membrane. The presence of this colored band indicates a positive result, while its absence indicates a negative result. The appearance of a colored band at the control region serves as a procedural control, indicating that proper volume of specimen has been added and membrane wicking has occurred.

3 - MATERIALS PROVIDED – PACKAGING

Product	Type	REF	Pack
Strep B Card	Immunochromatographic test	**VQ81305** (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 tubes with dropper tips Secondary packaging: cardboard box.
Strep B Card	Immunochromatographic test	**VQ81310** (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 tubes with dropper tips 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

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.
- Strep B 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.
- 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.
- Follow the normal precautions taken for laboratory reagents. Dispose of waste in accordance with local, regional or national regulations.
- 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. 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 freeze. Do not use beyond the expiration date.

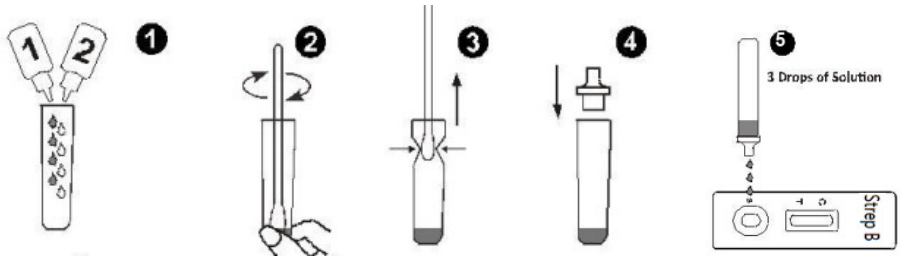
7 – Specimens Collection and Preparation

1. The quality of specimen obtained is of extreme importance. Collect swab specimens using standard clinical methods.
2. 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. Do not freeze. Swabs can be stored at room temperature up to 4 hours, or refrigerated (2-8°C) up to 24 hours. All specimens should be allowed to reach room temperature (15-30°C) before testing.
3. If a liquid transport method is desired, use Modified Stuart's Transport Media and follow the manufacture's instructions. Do not place the swab in any transport device containing medium. Transport medium interferes with the assay, and viability of organisms is not required for the assay. Do not use transport media formulas that include charcoal or agar.
4. If a **bacteria culture** is desired, lightly roll the swab on an appropriate cell culture plate **before** using it in the test. The extraction reagents in the test will kill bacteria on swabs and make them impossible to culture.

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. Best results will be obtained if the test is performed immediately after opening the foil pouch.

1. Add 5 drops of Extraction Solution 1 (light red, about 250 µL) and 5 drops of Extraction Solution 2 (about 250 µL) in a testing tube (the solution should turn light pink (colourless)), immediately put the swab into the tube. (1)
2. Mix the solution by rotating the swab forcefully against the side of the tube at least 2 minute. Best results are obtained when the specimen is vigorously extracted in the solution (2). 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 (3). Discard the swab.
3. Remove the test card from the sealed pouch just before using it.
4. Fit the dropper tip on top of the extraction tube (4). Dispense exactly 3 drops (about 150 µL) from the testing tube, into the specimen well of the test cassette (5). Start the timer.
5. **Read the results at 10 minutes.** Do not interpret test after 20 minutes.



9 - Reading and interpretation

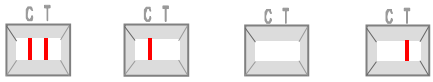
Interpret the results as follow:

NEGATIVE: The presence of only one pink-red color band within the result window indicate negative result.

POSITIVE: The presence of two color bands ("T" and "C" bands) within the result window regardless of which band appears first indicates a positive result.

Note: Generally, the higher the analyte level in the specimen, the stronger the "T" band color will be. When the specimen analyte level is close to but still within the sensitivity limit of the test, the color of the "T" band will be very faint.

INVALID: After performing the test and no pink-red control color band is visible within the result window, this result is considered invalid. The directions may not have been followed correctly or the test may have deteriorated. It is recommended that the specimen be re-tested.



Positive Negative Invalid

10 - Internal quality control

Internal procedural control is included in the test. A red 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 red to light pink (colourless) as you adds Extraction Solution 2 to Solution 1. 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. UD80030).

12 - Performances characteristics

Clinical study. The Strep B CARD has been evaluated with specimens obtained from patients of STD clinics. Culture is used as the reference method for the Strep B CARD test. Specimens were considered positive if culture indicated a positive result. Specimens were considered negative if culture indicated a negative result.

Method		Culture		Totale results
Strep B Card Mascia Brunelli	Results	Positive	Negative	
	Positive	120	7	127
	Negative	6	360	366
Total results		126	367	493

Relative Sensitivity: 95.2% (95%CI: *89.9%-98.2%)
Relative Specificity: 98.1% (95%CI: *96.1%-99.2%)
Accuracy: 97.4% (95%CI: *95.5%-98.6%)

Cross-reactivity – Intra-Inter assay: Within-run and Between-run precision have been determined with three different lots by using Strep B negative, low, middle and high positive specimens. Ten replicates of each level were tested each day for 3 consecutive days. The specimens were correctly identified >99% of the time.

Cross-reactivity

Cross-reactivity with other organisms has been studied using suspensions of 10^7 UFC/test. The following organisms were found negative when tested with the Strep B CARD

Acinetobacter calcoaceticus	Pseudomonas aeuroginosa	Proteus mirabilis
Acinetobacter spp	Gadnerella vaginalis	Chlamydia trachomatis
Enterococcus faecalis	Salmonella choleraesius	Streptococcus group A/C
Enterococus faecium	Candida albicans	Haemophilus influenzae
Staphylococcus aureus	Proteus vulgaris	Klebsiella pneumoniae

13 - LIMITATIONS OF THE METHOD

- The Strep B Card is for professional in vitro diagnostic use, and should only be used for the qualitative detection of Group B Streptococcus. No meaning should be inferred from the color intensity or width of any apparent bands.
- The accuracy of the test depends on the quality of the swab specimen. False negatives may result from improper specimen collection or storage. A negative result may also be obtained from patients at the onset of the disease due to low antigen concentration.
- The test does not differentiate asymptomatic carriers of Group B Streptococcus from those with infection. If clinical signs and symptoms are not consistent with laboratory test results, a follow-up cell culture is recommended.
- 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.
- In order to increase the sensitivity we suggest this protocol for the preanalytical phase:
  - remove swab from transport medium
  - Inoculate swab in selective broth medium such as Todd-Hewitt CNA (known also as LIM Broth) (Biolife/Mascia Brunelli REF. 552134B)
  - Incubate for 18-24 hours at 37C° in ambient air with 5% CO_2.

14 - REFERENCES

1. Finch, R.G., French, G.L., and Philips, I.; Group B streptococci in the female genital tract; Br. Med. J., 1 (6020) 1245-1247, 1976
2. You, M.D., Mason, E.O., Leeds, L.J., Thompson, P.K., Clark, D.J. and Gardner, S.E.; Ampicilin prevents intrapartum transmission of group B streptococcus; JAMA 241 (12) 1245-1247 ,1979
3. Boyer, K.M., and Gotoff, S.P.; Prevention of early-onset neonatal group B streptococcal disease with selective intrapartumchemotaxis; N. Eng. J. Med. 314 1665-1669, 1986
4. Lim, D.V., Morales, W.J., Walsh, W.J. and Kazanis, D.; Reduction of morbidity and mortility rates for neonatal group B streptococcal disease through early diagnosis and chemoprophylaxis; J. Clin. Microbiol. 23 489-492, 1986

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 11	Updated layout and content; alignment to the Italian version revision index	2023/08

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

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