

**Strep B Test
014B255**

**A rapid, one step test for the qualitative detection of Strep B antigen
in vaginal and cervical swab specimens
For professional *in vitro* diagnostic use only.**

INTENDED USE

The Strep B Antigen Test Card is a rapid diagnostic immunoassay for the direct qualitative detection of group B streptococcus (GBS) antigen from vaginal and cervical swab specimens, to aid in the diagnosis of streptococcus B infection. The assay may also be used as a confirmatory test for colonies of Group B streptococci grown on agar plates. This test is an *in vitro* diagnostic test for healthcare professional use only.

SUMMARY

Group B streptococci (GBS) are among the most frequent causes of life-threatening infections in neonates. Between 5% and 30% of all pregnant women are colonized with GBS¹. Several recent studies have shown that the intrapartum treatment of GBS-colonized women significantly reduces the incidence of GBS-caused sepsis.²⁻⁴ Therefore, screening for GBS is important. 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.

Strep B Antigen Test Card is a simple, rapid and sensitive immunochromatographic assay for screening of GBS antigen from patient vaginal or cervical swab specimens. The test procedure takes less than 20 minutes and does not require special instrumentation.

TEST PRINCIPLE

The Strep B Antigen Test Card utilizes the chemical extraction of a GBS-specific antigen from the bacteria followed by the immunochromatographic assay for qualitative detection of GBS.

In the test procedure, a unique set of polyclonal antibodies are employed. One antibody is immobilized on a porous membrane while the other antibody is conjugated to dye particles as signalling component. A swab specimen from a patient is treated with Extraction Reagent to extract the antigen. The liquid extract is applied and allowed to migrate through the absorbent area and along the membrane. If GBS antigen is present, the labeled antibody-dye conjugate binds to it, forming antibody-antigen complex. As the mixture flows along the membrane, the complex is captured by the antibody immobilized in the test (marked "T") zone of the membrane, producing a visible rose-pink color band. Unbound dye-conjugated reagent is captured by the antibody immobilized in the control (marked "C") zone of the membrane. A rose-pink line in the test zone indicates the presence of the GBS antigen. A rose-pink line in the control zone indicates that the test is working properly. When only a control line appears with no test line, the GBS antigen has not been detected and the test result is interpreted as negative. The control line gives an added measure of quality control by demonstrating antibody recognition; assuring that the procedure was performed correctly and that the reagents are still chemically active. A desiccant is enclosed with the Testing Device to stabilize the incorporated biomolecular reagents.

MATERIALS

Materials Provided

- 25 Strep B Antigen Test Cards (per test kit)
- 25 disposable dropper (inside pouch)
- Extraction reagent (0.01% sodium azide)
- Positive Control (non-viable group B streptococci)
- 25 extraction cups
- 1 cup holder
- 25 swabs
- 1 package insert

Materials Required But Not Provided

- Timer

STORAGE AND STABILITY

The positive control should be refrigerated (2-8°C). The **Strep B Antigen Test Card** and other components can be stored in the sealed pouch at room temperature or refrigerated (2-28°C) and used until the expiry date printed on the pouch. The test device must remain in the sealed pouch until use.

- Do not freeze.
- Do not use after expiration date.

PRECAUTIONS

- For professional *in vitro* diagnostic use only.
- Do not use test card or reagents after their expiration date.
- Do not touch the swab tip at any time.
- Do not use if pouch is torn or damaged.
- Do not allow a sample swab to come in contact with any reagent vial dropper tip. Reagent or bacterial contamination may result.
- Use only the sterile swabs provided. Swabs from any other source may give erratic results.
- Use protective laboratory clothing, disposable gloves and protective glasses while handling specimens.
- All samples should be considered as if they contain infectious agents and handled with the corresponding precautions.
- Discard the used test device, specimen and gloves following the standard procedures for proper disposal of microbiological agents.
- Wash and disinfect your hands after taking the test.

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SPECIMEN COLLECTION AND PATIENT PREPARATION

- The vaginal and cervical test specimens should be collected by a standard swab collection method. Grossly bloody samples should be avoided.
- Patient swab may be stored or transported in a closed tube at 2-8°C for up to 7 days before testing, either without media or with Modified Stuart's media (1 ml or less). Do not freeze the swabs.
- To use Strep B Antigen Test Card as culture confirmation assay, collect two or three 24-hour colonies from an agar plate with a test swab. The test procedure is the same for all swab specimens.

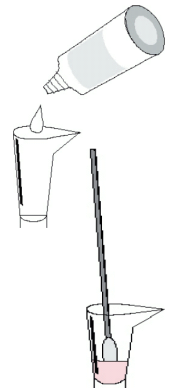
DIRECTIONS FOR USE

Procedure Notes

1. Bring all specimens and controls to room temperature (15°-28°C) before testing.
2. Do not open the protective foil pouch until ready to perform the test.
3. Do not use commercial controls other than those included with the kit. They may contain additives which will interfere with test performance.

A. Extraction of GBS Antigen from Sample Swab

1. Label a Test Cup for each patient identification and place it in the Cup Holder.
2. Add 12 drops of Extraction Reagent to the Test Cup. Place the specimen swab in the Test Cup and twirl briefly to mix ingredients. Leave the swab in the cup at room temperature for at least five minutes, but no longer than 30 minutes.
3. Twirl the swab vigorously for a few seconds, then 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. The swab extract can be tested any time within the next 60 minutes by following the procedures outlined in the "Immunoassay of the Extract" section.

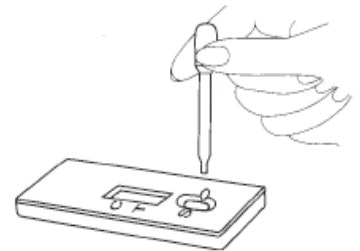


B. Control Testing Procedure

1. Add 12 drops of Extraction Reagent and one drop of Positive Control to the Test Cup.
2. Swirl the cup gently to mix contents and leave for five minutes at room temperature.
3. Complete the test according to the "Immunoassay of the Extract" section below.

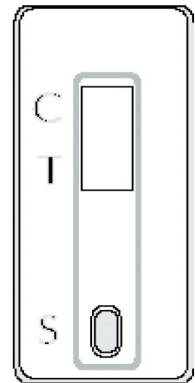
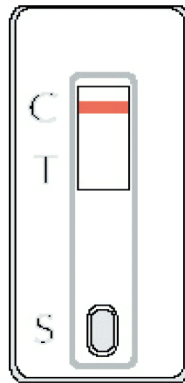
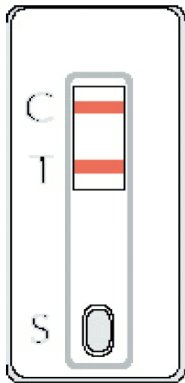
C. Immunoassay of the Extract

1. Remove a Testing Device and a Sample Dropper from the foil pouch. Discard the foil pouch and desiccant. Place the device on a level surface.
2. Using the Sample Dropper, add exactly five drops of the liquid extract from the Extraction Cup to the well marked "S" of the Testing Device.
3. Read the test results at 10 minutes.



IMPORTANT: To avoid incorrect readings, do not interpret the test results after more than 10 minutes.

INTERPRETATION OF RESULTS



Negative: One coloured line appears in the Control Zone ("C"), with no apparent color band in the Test Zone ("T"). GBS antigen is not present in the test sample at the level of sensitivity of the test.

Positive:* Two coloured lines appear. One in the Control Zone ("C") and one in the Test Zone ("T"). GBS-specific antigen was detected and the sample should be considered positive for GBS.

Invalid: The red line in the control region („C“) does not appear. Even if red lines appear in the test regions the result is not valid. In this case the specimen should be retested with a new test card.

Note: The intensity or width of the red lines may vary and are not important for the test interpretation. Even a faint pink line should be considered as positive.

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QUALITY CONTROL

Internal Controls

Strep B Antigen Test Card contains built-in quality control features. Distinctive coloured line in control zone indicates that the test was performed correctly, and that all test components were in good functional condition.

External Controls

Good laboratory practice recommends the use of control reagents. Positive Control containing GBS antigen is provided in the test kit to verify test performance.

LIMITATIONS

- 1 The test is limited to detection of GBS antigen in patient swab specimens or from colonies grown on agar plates.
- 2 For *in vitro* diagnostic use only.
- 3 Although the test is very accurate, a low incidence of false results may occur.
- 4 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.
- 5 The sensitivity (limit of detection) value of the test is 5×10^5 CFU (colony forming units) per ml on the 50 μ l test sample. Positive results are obtained when the amount of GBS antigen in the test corresponds to this value or above. Conversely, negative results are obtained when the amount of the antigen is below the sensitivity of the test.

PERFORMANCE CHARACTERISTICS

Relative Sensitivity and Specificity

The performance of Strep B Antigen Test Card was compared to that of a conventional culture method in a study of 101 clinical specimens. Vaginal and cervical swab specimens were obtained during routine examinations at physician's offices and in outpatient clinical laboratories. The specimens were evaluated by assaying with Strep B Antigen Test Card and by a conventional culture method.

The results of the comparative study are shown below.

Culture Method	Pos. (50) Neg. (51)	Strep B Antigen Test Card	
		Positive	Negative
		46	4
		5	46

Positive Negative
Culture Pos. (50) 46 4
Method Neg. (51) 5 46

Based on the comparison with a conventional culture method for detection of GBS from swab specimens, relative sensitivity of Strep B Antigen Test Card is 92.0% (46/50), and relative specificity is 90.2% (46/51).

Analytical Sensitivity

Strep B Antigen Test Card allows for the detection of GBS of serotypes Ia, Ib, Ic, II and III. A study of analytical sensitivity of the test was carried out by culturing serial dilutions of a logarithmic phase GBS broth culture, and by assaying the serial dilutions with three different lots of Strep B Antigen Test Card reagents. Comparing the colony counts of the cultures and the assay results yielded the sensitivity (limit of detection) value of 5×10^5 CFU (colony forming units) per ml, or 2.5×10^4 CFU/test, based on the 50 μ l test sample.

Cross-Reactivity

Cross-reactivity studies were performed with a variety of non-GBS microorganisms. The microorganisms listed below tested negative at 1×10^8 CFU/test.

Candida albicans (ATCC 14053)
Corynebacterium diphtheriae (ATCC 9015)
Escherichia coli (ATCC 25922)
Haemophilus influenzae (ATCC 35056)
Klebsiella pneumoniae (ATCC 13883)
Neisseria gonorrhoeae (ATCC 9826)
Pseudomonas aeruginosa (ATCC 27853)
Staphylococcus aureus (ATCC 29213, 25923)
Staphylococcus epidermidis (ATCC 12228)
Streptococcus, group A (ATCC 19615)
Streptococcus, group C (ATCC 12388)
Streptococcus, group D (ATCC 12389)
Streptococcus, group F (ATCC 12393)
Streptococcus, group G (ATCC 12394)
Streptococcus pneumoniae (ATCC 9163, 6303, 10015)

Reproducibility

In a study of reproducibility of the test, serial dilutions of a GBS broth culture in a logarithmic growth phase were used to prepare masked and coded specimen swabs. The dilutions were also assayed by colony counting. Strep B Antigen Test Card assays of the swabs were performed at three separate test sites, using three different lots of the test reagents. Specimens obtained from

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dilutions below the detection limit (5×10^5 CFU/ml) tested negative in all assays. Specimens at or above the detection limit ($>5 \times 10^5$ CFU/ml) tested positive in all assays. The above results demonstrate excellent reproducibility of the Strep B Antigen Test Card.

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