

HEALTH-CHEM DIAGNOSTICS LLC

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ONE STEP STREP B™ SCREEN

NAME AND INTENDED USE

The **One Step Strep B™ Screen** is a rapid diagnostic immunoassay for the 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 performed on hemolytic colonies of streptococci grown on agar plates, as a confirmatory test.

SUMMARY

Group B streptococci (GBS) are among the 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.

One Step Strep B™ Screen 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.

PRINCIPLE OF THE TEST

HCD'S **One Step Strep B™ Screen** utilizes the chemical extraction of a GBS-specific antigen from the bacteria followed by the immuno-chromatographic assay for qualitative detection of GBS.

In the test procedure, polyclonal and monoclonal antibodies are employed. One set of antibodies is immobilized on the porous membrane while the other antibodies are conjugated to dye particles as signaling components. A swab specimen from a patient is treated with Extraction Reagent to extract the antigen. The liquid extract migrates through the absorbent area along the membrane. If the 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 zone of the membrane, producing a visible magenta (reddish-purple) color band. Another dye-conjugated reagent is captured by the antibody immobilized in the control zone of the membrane.

A magenta (reddish-purple) line in the test zone indicates the presence of the GBS antigen. A magenta (reddish-purple) line in the control zone indicates 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 considered negative.

The control line is designated as internal control of antibody interactions, proper test procedure, and activity of reagents. A desiccant is enclosed with the Testing Device to stabilize the incorporated bimolecular reagents.

REAGENTS AND MATERIALS PROVIDED

1. HCD'S **One Step Strep B™ Screen**
2. Extraction Reagent (10.0 ml)

MATERIALS REQUIRED BUT NOT PROVIDED

1. Clock or timer
2. Test tube*
3. Sample swab*
4. Positive control*

* These products are available in bulk and/or when packages of specified number of tests are ordered.

STORAGE

Store the HCD'S **One Step Strep B™ Screen** strip at 2° - 8°C; do not freeze. Refer to the expiration date for stability.

WARNINGS AND PRECAUTIONS

1. Wear gloves while handling specimens.
2. Dispose of gloves and specimens using good microbiological practices.
3. Do not touch the swab tip at any time.
4. Wash hands after performing the test.
5. Use only the sterile plastic-shafted dacron swabs.
6. Do not allow a sample swab to come in contact with any reagent vial dropper tip.
7. Do not use reagents after their expiration dates.

SPECIMEN COLLECTION AND STORAGE

1. The vaginal and cervical test specimens should be collected by a standard swab collection method. Grossly bloody samples should be avoided.
2. 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.
3. To use HCD'S **One Step Strep B™ Screen** as a culture confirmation assay, collect two to three 24-hour colonies from an agar plate with a test swab. The test procedure is same for all swab specimens.

Test Procedure

NOTE: Read all test instructions before running patient samples or controls.

Procedure Notes

1. Bring all specimens and controls to room temperature (18°- 25°C) before testing.
2. Do not open the protective foil pouch until ready to perform the test.

A. Extraction

1. Label a test tube for each patient identification and place in the test tube holder.
2. Add 12 drops of Extraction Reagent to the test tube. Place the specimen swab in the test tube and twirl briefly to mix ingredients. Leave the swab in the test tube at room temperature for at least five minutes, but no longer than thirty 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 immediately or within 60 minutes.

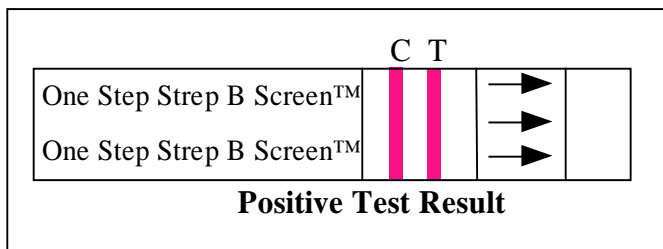
B. Immunoassay of the Extract

1. Remove the HCD'S **One Step Strep B™ Screen** from the foil pouch. Discard the foil pouch and desiccant.
2. Place the sample end of the test strip directly into the test tube. Be careful not to submerge the dipstick below the "maximum level" line indicated by the arrows.
3. Read the results at 10 minutes.

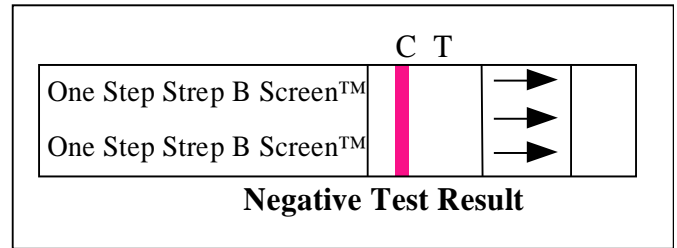
IMPORTANT: to avoid incorrect readings, do not interpret the test results after more than 10 minutes.

INTERPRETATION OF RESULTS

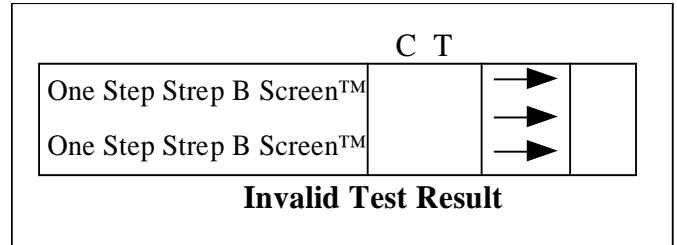
1. **Positive.** Two magenta (reddish-purple) bands appear: one in the test zone and one in the control zone. GBS-specific antigen is detected, and the sample should be considered positive for GBS.



2. **Negative.** One magenta (reddish-purple) band appears in the control zone, with no band in the test zone. GBS antigen is not present in the test sample at the level of sensitivity of the test.



3. **Invalid.** At the end of the assay, there are no color bands on the membrane, or a band is visible in the test zone but not in the control zone. The test is invalid. The specimen should be retested using a new HCD'S **One Step Strep B™ Screen** strip.



QUALITY CONTROL

A. Internal Controls:

The HCD'S **One Step Strep B™ Screen** contains built-in quality control features. Distinctive magenta (reddish-purple) band in the control zone indicates that the test was performed correctly, and that all test components were in good functional condition.

LIMITATIONS OF THE PROCEDURE

1. The test is limited to detection of GBS antigen in patient swab specimens and in beta-hemolytic cultures 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 a physician after all clinical and laboratory findings have been evaluated. Cases in which patient sample tests negative while the clinical symptoms are indicative of the infection should be investigated further.
5. The sensitivity of the HCD'S **One Step Strep B™ Screen** is 2.5×10^4 CFU (colony forming unit) of GBS per test. 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 HCD'S **One Step Strep B™ Screen** was compared to that of a conventional culture method in a study of 184 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 HCD'S **One Step Strep B™**

Screen and by a conventional culture method.

Prior to performing the assay, each swab was used to inoculate a sheep blood (Trypticase) soy agar plate for anaerobic culture at 37°C. After 24 hours the plates were examined for beta-hemolytic colonies; those without beta-hemolytic colonies were re-examined after 24 more hours of culture. Beta-hemolytic streptococcal colonies were grouped with commercially available latex streptococci grouping tests.

The results of the comparative study are shown below.

HCD'S One Step Strep B™ Screen			
		Positive	Negative
Culture	Positive (69)	67	2
Method	Negative (115)	2	113

Based on the comparison with a conventional culture method for detection of GBS from swab specimens, relative sensitivity of HCD'S **One Step Strep B™ Screen** is 97.1% (67/69), and relative specificity is 98.2% (113/115).

Analytical Sensitivity

HCD'S **One Step Strep B™ Screen** allows 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 HCD'S **One Step Strep B™ Screen** 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 $\geq 1 \times 10^8$ CFU/test.

- *Candida albicans* (ATCC 14053)
- *Corynebacterium diptheriae* (ATCC 9015)
- *Escherichia coli* (ATCC 25922)
- *Haemophilus influenzae* (ATCC 35056)
- *Klebsiella pneumoniae* (ATCC 13883)
- *Neisseria gonorrhoea* (ATCC 9826)
- *Pseudomonas aeruginosa* (ATCC 27853)
- *Staphylococcus aureus* (ATCC 29213 & 25923)
- *Staphylococcus epidermidis* (ATCC 12228)
- *Streptococcus* Group B (ATCC 12386)
- *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. HCD'S **One Step Strep B™ Screen** assays of the swabs were performed at three separate test sites, using three different lots of the test reagents. Specimens obtained from dilutions below the detection limit ($\geq 5 \times 10^4$ CFU/ml) tested negative in all assays. Specimens from dilutions near the detection limit ($\approx 1 \times 10^6$ CFU/ml) tested positive in all assays. The above results demonstrate excellent reproducibility of the HCD'S **One Step Strep B™ Screen**.

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