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In vitro Diagnostic Reagents
Ref. (08AM) No. 209

B Strept AD "SEIKEN"

Group B Streptococcal Antigen Detection Kit by
Slide Latex Agglutination

For 50 tests

INTRODUCTION

Streptococci are divided into 20 groups (Lancefield's group A through H, K through V) based on the carbohydrate antigens present in their cell wall. Some are important human pathogens, including group B streptococci (GBS: *Streptococcus agalactiae*), which are a major cause of severe neonatal infections, including sepsis, pneumonia and meningitis. Such infections, which result from the vertical transmission of GBS from mother to infant during labor or delivery, are classified into two types based on the timing of symptom development. In early-onset infections, symptoms typically develop within a few hours to a week after birth while in the late-onset type, one week or more typically passes before the appearance of symptoms. Since disease progression after onset is typically rapid and treatment ineffective, significant infant mortality and morbidity are not uncommon. To prevent such outcomes, the administration of antibiotics to colonized mothers prior to or during delivery should be considered.

While genital cultures obtained during pregnancy are useful in identifying colonized mothers, determining the level of colonization close to the delivery date is strongly recommended since levels are known to change over time. Furthermore, various studies, including those of Itakura et al, have shown a strong correlation with the level of GBS colonization at delivery and an increased risk of neonatal infection, with colonization levels of 10^6 CFU/mL or more placing infants at risk. In deliveries involving complications (eg., premature delivery, premature rupture of membranes, amnionitis, etc.) levels as low as 10^4 CFU/mL were also correlated with neonatal infection.

B STREPT AD "SEIKEN" was developed for the rapid detection of GBS in pregnant women just prior to delivery to accurately identify mothers at risk of passing on GBS infections to their infants. The kit is based on rapid slide latex agglutination and two protocols are employed. One is a direct test for speed and the other includes an enrichment step when increased sensitivity is desired. Total test times and sensitivities for the two procedures are 10 minutes (10^6 CFU/mL) and 3 hrs and 20 minutes (10^4 CFU/mL), respectively.

CHARACTERISTICS

1. Simple test procedure.
2. Rapid test procedure.
   (Direct method: 10 minutes; Enrichment method: 3 hrs and 20 min.)
3. Easy to interpret results.

CONTENTS

1. Sensitized latex — 2 mL x 1 vial
A suspension of latex particles sensitized with group B streptococcal antigen specific antibodies (rabbit, polyclonal) containing sodium azide as a preservative (0.1 w/v %).

2. Control latex — 2 mL x 1 vial
A suspension of latex particles sensitized with non-immune rabbit IgG antibody containing sodium azide as a preservative (0.1 w/v %).

3. Positive control — 1 mL x 1 vial
A suspension of inactivated group B streptococci (2×10^8 cells/mL) containing sodium azide as a preservative (0.1 w/v %).

4. Extraction Reagent 1 — 5 mL x 1 vial
An acetic acid solution

5. Extraction Reagent 2 — 5 mL x 1 vial
A sodium nitrite solution

6. Extraction Reagent 3 — 5 mL x 1 vial
A Tris buffer solution

7. Accessory reagents

Direct Method		Culture method		
Sample cups	55 pcs.	Enrichment broth	3 mL	50 pcs.
Droppers	55 pcs.	Sterile swabs		50 pcs.
Sterile swab	50 pcs.	Disposable slides		30 pcs.
Disposable slides	30 pcs.	Stirrers		120 pcs.
Stirrers	120 pcs.			

INTENDED USE

To detect group B streptococci from vaginal swabs as an aid in the prevention of neonatal GBS infections.

PRINCIPLE OF THE TEST

Latex particles sensitized with antibodies against group B streptococcal antigen will react in their presence to form a visible agglutination pattern. Antigen is extracted from whole cells with nitrite prior to adding the latex reagent.

PROCEDURES

This kit is used to directly detect GBS from vaginal swabs and from genital cultures following a short enrichment step. In non-emergency situations and when sufficient time is available, the more sensitive procedure using the enrichment step is recommended while direct testing should be performed in the case of emergency deliveries. Note, however, that in pregnancies involving complications, the more sensitive enrichment method is also recommended since the risk for GBS transmission is correlated with levels of colonization lower than for normal deliveries.

Reagent Validation

Although it is unnecessary to perform the positive control test each time the assay is run, it is recommended when confirming reagent performance, training new personnel to show agglutination patterns, etc.

To do so, place one drop of the positive control onto a cotton swab and perform the extraction steps as described in **B. Direct Method**. Agglutination in the sensitized latex but not in the control latex confirms reagent integrity.

A. Detection by Enrichment Method

1. Materials and reagents necessary for the test

37 °C water bath, micropipette and tips (50 μL), test tube holder, centrifuge capable of 900 g: 3,000 rpm or greater in bench-top type.

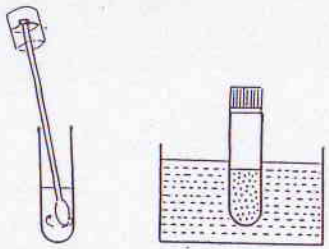
2. Reagent preparation

1) No reagent preparation is required.
2) Bring enrichment culture media to 37 °C before use.

3. Sample preparation

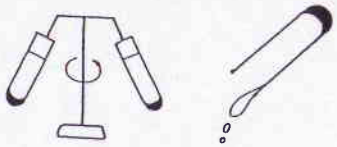
Vaginal specimens are obtained using the sterile swabs provided according to recommended sampling procedures.

4. Working procedure



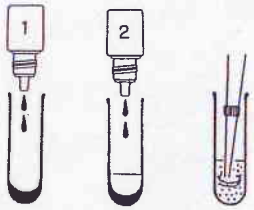
Culture

1) Place the sample-containing swab into the enrichment broth. Mix thoroughly and discard the swab.
2) After closing the cap, place the enrichment broth in the water bath and culture for 3 hrs.



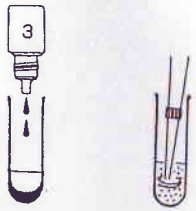
Centrifugation

1) Centrifuge the culture for 10 min. at 900 g.
2) Carefully discard the supernatant without disturbing the pellet.

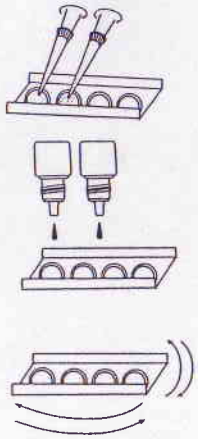


Antigen extraction

1) Place the culture tube into the test tube rack.
2) Add two drops of Extraction Reagent 1.
3) Add two drops of Extraction Reagent 2.
4) Mix well with a micropipette tip and let sit for 5 mins.



5) Add two drops of Extraction Reagent 3.
6) Mix well with a micropipette tip and use as sample extract.



Slide Latex Agglutination

1) With the micropipette place 50 μL of the sample extract into each of two circles of the slide agglutination card and spread evenly within the circle with the tip.
2) Place one drop of Sensitized latex next to the sample extract in the first circle and one drop of Control latex next to the sample extract in the second circle.
3) Using separate stirrers, mix the sample extract with the latex reagent so that the entire area is covered in each circle. Continue mixing for two minutes by gentry rotating and tilting the card back and forth.
4) Promptly read the results under suitable lighting.

B. Direct Detection Method

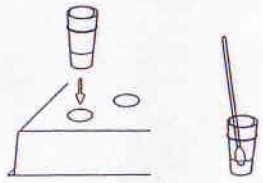
1. Reagent preparation

Use reagents as supplied.

2. Sample preparation

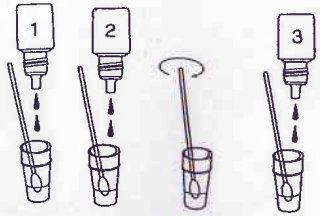
Vaginal specimens are obtained using the sterile swabs provided according to recommended sampling procedures.

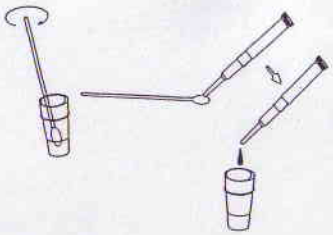
3. Working procedure



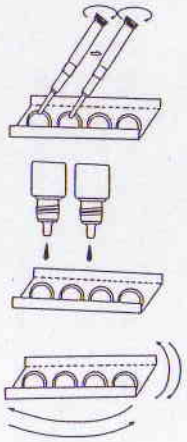
Antigen extraction

1) Place a sample cup into the sample cup holder.
2) Place the sample-containing swab into the sample cup as shown at the left. Note: for easier mixing and handling during subsequent steps, it is recommended that the swab head be removed by bending of the stick for several times.
3) Add two drops of Extraction Reagent 1.
4) Add two drops of Extraction Reagent 2.
5) Mix well with the edge of the swab tip and let stand for 5 mins.
6) Add two drops of Extraction Reagent 3.





7) Repeat step 5.
8) Carefully remove as much of the extract attached to the swab as possible using the dropper provided and add to the sample cup.



Slide Latex Agglutination

1) With the dropper place 2 drops of the sample extract into each of two circles of the slide agglutination card and spread evenly within the circle with the dropper.
2) Place one drop of Sensitized latex next to the sample extract in the first circle and one drop of Control latex next to the sample extract in the second circle.
3) Using separate stirrers, mix the sample extracts with the latex reagents so that the entire area is covered in each circle. Continue mixing for two minutes by gently rotating and tilting the card back and forth.
4) Promptly read the results under suitable lighting.

NOTES

1. When obtaining vaginal samples, be sure to only use the sterile swabs provided in the kit.
2. Similarly, when performing the enrichment detection method, only use the enrichment broth provided. Check the media for turbidity, the presence of foreign or particulate matter, etc., before use.
3. When transporting samples, be sure to place the swab in the transport container. For the enrichment method, place the swab into the culture media as soon as possible after sampling.
4. In the direct method, the presence of excess mucous material on the swab may interfere with the nitrite extraction step, thus giving false negative results.
5. When performing the culture method, always incubate for the specified three hours as incubating for shorter times may reduce test sensitivity while longer times may increase the sensitivity of the test such that clinically insignificant false positives result.

INTERPRETATION

1. Refer to notes below when interpreting the results

Intense agglutination can be seen against a clear background	3+
Agglutination can be seen against a slightly turbid background	2+
Agglutination can be seen against a turbid background	1+
Homogenous white suspension with no visible agglutination	-

Agglutination patterns of 1+ or greater are considered positive

Note: Confirm the absence of agglutination in the control latex; if agglutination is seen, the results are indeterminate.

2. Precautions on interpretation

1) In a clinical study by Itakura, et al, it was reported that the risk of passing on infection was correlated with GBS colonization at levels of more than 10^4 CFU/mL in the cases of premature delivery, early leak of amniotic fluid, fever, etc., while in the case of non-complicated deliveries, the risk of neonatal infection was correlated with GBS levels of more than 10^6 CFU/mL. On the basis of this and other clinical studies, the detection limits of this kit were set at 10^6 CFU/mL for the direct method and 10^4 CFU/ml for the enrichment method, respectively.
2) Although the above detection limits for the assays were set as indicated above, it is possible that specimens containing GBS at concentrations lower than the detection limit may give positive results.
3) It should be noted that neonatal infections may result from GBS colonization levels which are below the detection limit of this kit.

PERFORMANCE

1. Sensitivity

When the standard strain DK-ST-2 was grown in Todd-Hewitt broth at 35 °C for 18 hours and resuspending in saline (OD 650 = 0.4), followed by heat treatment at 20 minutes at 121 °C, 100 μL samples of 3-fold serial dilutions were detected at a dilution up to $1:3^3$ with this kit.

2. Specificity

When the various *Streptococcus* strains listed below were cultured in Todd-Hewitt broth for 18 hrs at 35 °C and tested with this kit, only group B streptococci gave positive results.

Strain		Strain	
Group A streptococci	DK-ST-1	Group F streptococci	DK-ST-6
Group B streptococci	DK-ST-2	Group G streptococci	DK-ST-8
Group C streptococci	DK-ST-3	Group H streptococci	DK-ST-9
Group D streptococci	DK-ST-4	Group I streptococci	DK-ST-10
Group E streptococci	DK-ST-5	Group J streptococci	DK-ST-11

All strains were provided by the Japanese NIH.

3. Reproducibility

When the test described in **1. Sensitivity** above was performed 5 times, all test specimens were detected at the $1:3^3$ dilution.

4. Clinical Trial

In clinical trials using vaginal samples, the detection limit of this kit was determined to be 10^6 CFU/mL by the direct and 10^4 CFU/mL by the enrichment method.

The correlation between the direct and quantitative culture method and between the enrichment and quantitative method were as follows:

Correlation Between Enrichment Detection Method and Quantitative Culture

		CULTURE (CFU/mL)		
		$\geqq 10^4$	$<10^4$	Total
B STREPT AD "SEIKEN" Enrichment	POS	52	22*	74
	NEG	0	1126	1126
	Total	52	1148	1200

Sensitivity: 100 %
Specificity: 98.1 %
Agreement: 98.2 %

Predictive value of a
Positive result: 70.3 %
Negative result: 100 %

(The above numbers indicate the number of samples)
* They contains 20 GBS positive samples at a level of less than 10^4 CFU/mL.

Correlation between Direct Detection Method and Quantitative Culture

		CULTURE (CFU/mL)		
		$\geqq 10^6$	$<10^6$	Total
B STREPT AD "SEIKEN" Direct	POS	30	16*	46
	NEG	4	2052	2056
	Total	34	2068	2102

Sensitivity: 88.2 %
Specificity: 99.2 %
Agreement: 99.0 %

Predictive value of a
Positive result: 65.2 %
Negative result: 99.8 %

(The above numbers indicate the number of samples)
* They contains 8 GBS positive samples at a level of less than 10^6 CFU/mL.

PRECAUTIONS

1. General

1) This kit is intended for in-vitro diagnostic use only.
2) Test results obtained by this kit should only be used as an indicator for the risk of neonatal GBS infection. Other relevant clinical symptoms and clinical history should be taken into account before taking preventative measures such as the prophylactic administration of antibiotics.

2. Handling precautions

1) Before using the sterile swabs and the transport containers provided, confirm the integrity of each piece, making sure that there are no tears in packaging, cracks, stains, etc.
2) Although visible bacterial growth may not be seen after performing the enrichment culture step, testing should still be carried out. Also, the extraction step should always be performed since the enrichment step is non-selective and may allow the growth of other bacteria, resulting in nonspecific agglutination.

3) There are reports that some members of the genus *Escherichia* and *Candida* may possess antigens which cross-react with group-B specific antigens. Accordingly, such strains may cause false positive results if present in significant numbers.
4) If any of the extraction reagents come in contact with the skin, eyes or mouth, wash the affected area with copious amounts of water. Treatment by a physician may be required in severe cases.
5) Allow the reagents to come to room temperature before use. Do not freeze the reagents.
6) Mix the latex reagents by gently inverting several times before use.
7) Wipe the tip of the reagent bottle with a clean tissue before use. When adding the reagents, hold the bottle in a vertical position.
8) Do not interchange the caps of the reagent bottles.
9) Do not use the kit components for uses other than those indicated here.
10) Do not interchange or mix reagents with different lot numbers.
11) Always perform the test according to the kit instructions. Using kit reagents in a manner not recommended by this kit insert may give erroneous results.

3. Disposal precautions

1) The sensitized latex, control latex and positive control antigen contain sodium azide. As sodium azide may react with lead and copper piping to form highly explosive metal azides, dispose of by flushing with copious amounts of water.
2) Sterilize all sample-containing swabs, post-use culture media, and all other equipment used in performing this test by one of the following methods:
   1) Soaking in 2 w/v % glutaraldehyde for 1 hour or longer
   2) Soaking in 0.1 w/v % sodium hypochlorite (effective chlorine 1000 ppm or more) for 1 hour or longer
   3) Autoclaving at 121 °C for at least 20 minutes.

STORAGE AND SHELF LIFE

Storage : 2 - 10 °C protected from light (Do not freeze).
Shelf life: Up to the expiry date on the label.
(Note: Store accessory reagents at room temperature away from direct sunlight.)

PACKAGE

B Strept AD "SEIKEN"	Enrichment Detection Method	50 tests	~~(Product code: 230522)~~
B Strept AD "SEIKEN"	Direct Detection Method	50 tests	~~(Product code: 230515)~~
Also available			
B Strept AD "SEIKEN"	Culture Media	50 tests	~~(Product code: 230539)~~

REFERENCES

1) Lancefield, R. C., et al.: A serological differentiation of human and other groups of hemolytic streptococci. J. Exp. Med.,57,571 (1933).
2) El Kholy, A., et al.: Serological identification of group A streptococci from throat scraping before culture. J. Clin. Microbiol., 8,725 (1978).