BBL™STREPTOCARD™ ACID LATEX TEST
A STREPTOCOCCUS GROUPING TEST USING NITROUS ACID EXTRACTION FOR THE RAPID IDENTIFICATION OF β-HEMOLYTIC STREPTOCOCCI OF LANCEFIELD TYPES A, B, C, F AND G.

I. INTENDED USE

The **BBL™ Streptocard™** Acid Latex Test is a latex test system for the qualitative identification of Lancefield streptococcal groups A, B, C, F and G. The test is intended for use with streptococcus colonies that are β-hemolytic on blood agar.

II. SUMMARY AND EXPLANATION

β-hemolytic streptococci can be differentiated into Lancefield groups based on specific carbohydrate antigens.[1] Differentiation is necessary for clinical treatment and for epidemiological purposes.[2] For extraction of the group specific antigen prior to grouping a variety of methods have been used including hot acid,[1] hot formamide[3] and enzyme extraction methods.[4,5] The **BBL™ Streptocard™** Acid Latex Test is based on modified nitrous reagents,[6,7] which will rapidly extract the group antigens without the need for any incubation.

III. PRINCIPLE OF THE PROCEDURE

The **BBL™ Streptocard™** Acid Latex Test latex particles are sensitized with group specific antibody and will agglutinate in the presence of homologous antigen. The group specific antigens are extracted from streptococci by using an instant room temperature nitrous acid extraction procedure. The extract is then neutralized and the antigens are identified by agglutination.

IV. REAGENTS

Extraction Reagent 1	1 x 8.0 mL	Contains sodium nitrite solution with a pH indicator, with 0.1% sodium azide (preservative).
Extraction Reagent 2	1 x 8.0 mL	Contains 0.4N hydrochloric acid.
Extraction Reagent 3	1 x 8.0 mL	Contains neutralizing solution, with 0.1% sodium azide (preservative).
Test Latex A	1 x 2.5 mL	Test Latex A, B, C, F, and G consist of blue
Test Latex B	1 x 2.5 mL	latex particles sensitized with rabbit antibody
Test Latex C	1 x 2.5 mL	to appropriate group specific antigen, sus-
Test Latex F	1 x 2.5 mL	pended in buffer with 0.1% sodium azide
Test Latex G	1 x 2.5 mL	(preservative).
Control +	1 x 1.0 mL	Positive Control containing extracted antigen from streptococcal groups A, B, C, F and G with 0.1% sodium azide as a preservative.

Reaction Cards	50	Disposable; 6 reaction circles per card.
Mixing Sticks	250	Disposable.

Precautions: For *in vitro* Diagnostic Use.

CAUTION: This product contains Natural Rubber Latex, which may cause allergic reactions in some individuals**.**

Do not use test components beyond the expiration date.

Pathogenic microorganisms including Hepatitis B Virus and Human Immunodeficiency Virus may be present in specimens. "Universal Precautions"[15,16] and institutional guidelines should be followed in handling all items contaminated with blood or other body fluids. Extraction Reagents do not always render bacteria nonviable. After use, contaminated materials must be sterilized by autoclaving.

WARNING: Contact with combustible material may cause fire. Keep away from combustible material. Toxic if swallowed. When using kit, do not eat or drink. Wear suitable protective clothing, gloves and eye/face protection.. In case of accidental exposure or if you feel unwell, seek medical advice immediately.

Reagents contain sodium azide. Very toxic by inhalation, in contact with skin, and if swallowed. Contact with acids liberates very toxic gas. After contact with skin, wash immediately with plenty of water. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide build-up.

REAGENTS: Extraction Reagents 1 and 2 contain a mild irritant and a weak acid respectively. Avoid direct contact by wearing suitable protective equipment. If the material comes into contact with the skin, mucous membranes or eyes immediately wash the area by rinsing with plenty of water.

Do not allow reagents to become contaminated by allowing the dropper tip to touch the specimen on the reaction card. Ensure caps are securely fitted on reagent bottles after each use to prevent contamination and drying out of the reagents.

CARDS: Care should be taken not to finger-mark the test areas, since this may result in an oily deposit and improper test results.

Storage: Upon receipt, store kit at 2-8°C. DO NOT FREEZE. Under these conditions the reagents will retain their reactivity until the date shown on the bottle labels. After use return the kit to the refrigerator, storing bottles in an upright position.

Product Deterioration: Do not use the kit if the Control + does not yield appropriate results. Refer to "User Quality Control." Examine the Control + and Extraction Reagents for evidence of contamination, evaporation or other signs of deterioration, such as turbidity. Examine the Test Latex, and do not use if not homogeneously suspended.

V. SPECIMEN PREPARATION

Samples for identification should be grown on a blood agar plate 16 - 24 h at 35 ± 2°C. Note the hemolytic reaction of suspect colonies. It is also advisable to perform a Gram stain and catalase test to confirm the presence of Gram-positive, catalase-negative cocci. For further details, please consult standard references.[2]

Organisms of groups A, B, C, F or G are normally β-hemolytic. If an α-hemolytic or non-hemolytic organism appears to belong to one of these groups, the species identification should be confirmed by biochemical tests.

VI. PROCEDURE

Materials Provided: All materials as listed under "Reagents" and accessories.

Materials Required But Not Provided: Microbiological loop, Pasteur pipettes and glass or plastic 12 x 75 mm tubes. Also required are the necessary equipment and labware used for preparation, storage and handling of serologic specimens.

Test Method: If fewer tests than six are to be performed, the card may be cut with scissors and the unused portion saved for future use. The test area, all reagents, test specimens and test components should be at room temperature (15 - 30°C) when used.

1. Label one 12 x 75 mm test tube for each specimen to be tested.
2. Add 3 drops of **Extraction Reagent 1** to each specimen tube by holding the bottle and gently squeezing.
3. Add 3 drops of **Extraction Reagent 2** to each specimen tube. This will change the color of the solution from blue to orange/yellow.
4. Select 2 - 5 similar β-hemolytic colonies with a microbiological loop and emulsify in the extraction solution. If the culture is mixed avoid obvious contamination**. Do not use a swab**, because it will absorb too much of the liquid volume. If the colonies are small, use more than five and ensure that at least a slightly turbid suspension is obtained.
5. Add 3 drops of **Extraction Reagent 3** to each specimen tube. The solution color will now revert to a pale blue indicating the solution is neutralized.

   Note: If not assayed immediately, store the tube tightly capped at 2 - 8°C. Test within 24 h.
6. Dispense 1 drop from each Test Latex to be tested onto a separate circle on the reaction card.
7. Using a Pasteur pipette add 1 drop of extract to each of the five test circles.
8. With the mixing sticks provided, spread the mixture over the entire area of the circle, using a separate stick for each.
9. Gently rock the card manually for up to 1 min and observe for agglutination under normal lighting conditions. Read macroscopically; do not use magnification to aid reading.
10. Dispose of the reaction card in an appropriate biohazard container.

VII. INTERPRETATION OF TEST RESULTS

Positive Result: The test should be considered positive when agglutination occurs within 1 min with one Test Latex or when one Test Latex gives a substantially stronger reaction than the other four.

Negative Result: A negative result is obtained if no agglutination occurs after 1 min.

Granular or Stringy Reactions: Occasional granular or stringy reactions may be seen due to the particulate nature of the test material. When such reactions are seen to occur they should be interpreted using the following criteria:

- The result is **positive** when there is noticeable clearing of the blue background in the Test Latex.
- The result is **negative** when there is no noticeable clearing of the blue background in the Test Latex.

Uninterpretable Results: If more than one Test Latex strongly agglutinates, then the possibility exists that a mixed culture of streptococcal groups is present. Examine the plate and carefully select organisms of like morphology and retest. Subculture, if the suspected organism is overgrown or insufficient. If the reaction pattern is unaltered, re-isolate the organism or perform additional biochemical tests.

VIII. USER QUALITY CONTROL

Each lab should refer to the quality assurance plan established for their laboratory. Initially, upon receipt, the laboratory should check each shipment or lot of material prior to use to verify the performance of the product.

Streptococci of known group reactivity (See "Availability" for **BBL™ QualiSwab™** control strains) should be subjected to the complete test procedure. The performance of the test is assessed by the presence of agglutination in one latex suspension only, with the other four suspensions showing a negative (no agglutination) reaction for each reference strain tested. This will evaluate both the efficiency of the extraction procedure and the specificity of each reagent.

The positive control procedure and negative control procedure should be tested each day of use. Local, regional, or other laboratory regulations may apply which supercede package insert directions for frequency of testing the postive and /or negative control.

POSITIVE CONTROL- Shake each Test Latex and dispense 1 drop onto a separate circle of the card. Dispense 1 drop of Control + onto each of the five circles. Spread each mixture over the entire area of the circle, using a separate stick for each Test Latex. Rock the card manually for 1 min. Each of the five Test Latex suspensions should demonstrate obvious agglutination.

NEGATIVE CONTROL- Shake each Test Latex and dispense 1 drop onto a separate circle of the card. Prepare the Negative Control by adding extraction reagents to 12 x 75 mm test tube per steps 1, 2, 3 and 5 (Refer to section "Test Method" above). Dispense 1 drop of Negative Control

onto each of the five circles on a reaction card. Spread each mixture over the entire area of the circle, using a separate stick for each Test Latex. Rock the card manually for 1 min. No obvious agglutination should be evident for any Test Latex suspension.

The **BBL™ STREPTOCARD™ Positive Control** (catalog # 240965) contains a mixture of extracted antigen from streptococcal groups A, B, C, D, F and G with sodium azide as a preservative. This control may also be used to perform quality control when using the individual test latex reagents.

Patient results should not be reported if positive and negative controls do not yield appropriate results.

IX. LIMITATIONS OF THE PROCEDURE

False negative results can occur if an inadequate amount of the culture is used for extraction. Some streptococci, notably group F, produce minute colonies. When this occurs, use more colonies to prepare extract.

Nearly all the β-hemolytic streptococci isolated from human infections possess specific carbohydrate antigens, which can be recognized by serological reactivity. Attempts to extend these procedures to non β-hemolytic streptococci have been unsuccessful except for group B.[2]

Streptococcus pneumoniae share common antigenic determinant(s) with group C streptococci[8] and therefore may react with Test Latex C. *Streptococcus pneumoniae* colonies are typically α-hemolytic.

Certain strains of *Streptococcus milleri* possess A, C, F or G antigens and may therefore react with one or more of these Test Latex. *S. milleri* typically form minute and usually non-hemolytic colonies on blood agar plates. Identification of *S. milleri* may be performed using a scheme as that described by Lawrence *et al*.[9]

Streptococcus porcinus which is usually associated with swine, may react with Test Latex B. Differentiation with Group B streptococcus may be based on *S. porcinus* typically giving more pronounced zones of hemolysis and a positive pyrrolidonyl-arylamidase (PYR) test.[10]

Staphylococci and *Listeria monocytogenes* are β-hemolytic and can be distinguished from streptococci by their cellular morphology and catalase reactions.[11,12]

Some strains of group D streptococci have been found which also possess group G antigen.[13,14] For Group D *Enterococcus*, the group D component of these strains is not extracted efficiently by the reagents supplied with this kit and they should be identified using biochemical tests or the **BBL™ STREPTOCARD™** Enzyme Latex Test (See "Availability").

X. PERFORMANCE CHARACTERISTICS

The performance of the **BBL™ Streptocard™** Acid Latex Test was evaluated at three clinical sites. A total of 470 clinical isolates were tested. The results obtained with the **BBL™ Streptocard™ A**cid Latex Test were compared with test results obtained with other "commercially available latex kits" (Table 1).

Table 1

Organisms	# Tested	BBL™ Streptocard™ Acid Latex Test Sensitivity
Streptococci, Group A	111	111/111; 100%
Streptococci, Group B	120	117/120[1] ; 98%
Streptococci, Group C	83	80/83[2] ; 96%
Streptococci, Group F	66	59/66[3] ; 89%
Streptococci, Group G	90	90/90; 100%
Total	470	457/470; 97%

1. At one site, upon initial testing, 3 negative reactions were observed. Discrepancies were related to insufficient number of colonies used. Discrepancies were resolved upon retest.
2. At one site, upon initial testing, 3 negative reactions were observed. Discrepancies were related to insufficient number of colonies used. Discrepancies were resolved upon retest.
3. At one site, upon initial testing, 7 negative reactions were observed. Discrepancies were related to insufficient number of colonies used. 5 of the 7 discrepancies were resolved upon retest.

Overall Sensitivity of **BBL™ Streptocard™** Acid Latex Test = 97%.

Further testing was performed to demonstrate the specificity of the **BBL™ Streptocard™** Acid Latex Test using organisms other than β-hemolytic Streptococci (Table 2). All results of the **BBL™ Streptocard™** Acid Latex Test were compared to test results using other commercially available latex kits. These isolates demonstrated negative results in all cases.

Table 2

Organism	# Tested	BBL™ Streptocard™ Acid Latex Test Results
Alpha-hemolytic Streptococci	46	Negative
Non-hemolytic Streptococci	22	Negative
Staphylococcus aureus	23	Negative
Coagulase Negative Staphylococci	22	Negative
Group D Streptococcus (*Enterococcus* and Non-*Enterococcus* Group D)	61	Negative
Listeria	4	Negative

BBL™ Streptocard™Acid Latex Test demonstrated 100% specificity.

The performance of the **BBL™ Streptocard™** Acid Latex Test was tested with 24 ATCC™strains of streptococci (obtained from American Type Culture Collection, Rockville, MD, USA). Masked randomized specimens were tested on 3 consecutive days; 8 Group A, 6 Group B, 5 Group C, 2 Group F and 3 Group G. 100% agreement was observed. All streptococci were correctly grouped and there was no cross-reaction observed with the **BBL™ Streptocard™** Acid Latex Test.

XI AVAILABILITY

Cat. No.	Description
240951	**BBL™ Streptocard™** Acid Latex Kit, 50 Tests.
240950	**BBL™ Streptocard™** Enzyme Latex Kit, 50 Tests.
240960	**BBL™ Streptocard™** Test Latex A, one 2.5 mL bottle
240961	**BBL™ Streptocard™** Test Latex B, one 2.5 mL bottle
240865	**BBL™ Streptocard™** Test Latex C, one 2.5 mL bottle
240866	**BBL™ Streptocard™** Test Latex F, one 2.5 mL bottle
240867	**BBL™ Streptocard™** Test Latex G, one 2.5 mL bottle
240963	**BBL™ Streptocard™** Extraction Reagents 1, 2, & 3, box of one each 8 mL bottle.
240965	**BBL™ Streptocard™** Positive Control, one 1 mL bottle
249066	**BBL™ Streptocard™** Test Cards, box of 50
237059	**BBL™ QualiSwab**™ *S. pyogenes* Group A, ATCC™ 19615, one swab.
237056	**BBL™ QualiSwab**™ *Streptococcus* sp. Group B, ATCC™ 12386, one swab.
237018	**BBL™ QualiSwab**™ *Streptococcus* sp. Group C, ATCC™ 12388, one swab.
237019	**BBL™ QualiSwab**™ *Streptococcus* sp. Group F , ATCC™ 12392, one swab.
237020	**BBL™ QualiSwab**™ *Streptococcus* sp. Group G, ATCC™ 12394, one swab.

XII. REFERENCES

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TECHNICAL INFORMATION: In the United States, telephone B D Diagnostic Systems Technical Services, toll free (800) 638-8663

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