BBL™ STREPTOCARD™ ENZYME LATEX TEST
A LATEX AGGLUTINATION TEST FOR THE IDENTIFICATION OF STREPTOCOCCAL GROUPS A, B, C, D, F AND G.

I. INTENDED USE

The **BBL™ Streptocard™** Enzyme Latex Test is a latex test system for the qualitative identification of Lancefield streptococcal groups A, B, C, D, F and G.

II. SUMMARY AND EXPLANATION

Lancefield[1] showed that the majority of pathogenic streptococci possess specific carbohydrate antigens, which permit the classification of streptococci into groups. These streptococcal group antigens are extracted from the streptococcal cell wall in a liquid form, and reacted with group specific antibodies.

III. PRINCIPLE OF THE PROCEDURE

The **BBL™ Streptocard ™** Enzyme Latex Test latex particles are sensitized with group specific antibody and will agglutinate in the presence of homologous antigen. In the absence of such antigen, the latex particles will remain in a smooth suspension. The use of a patented enzymatic extraction in the **BBL™ Streptocard ™** Enzyme Latex Test procedure considerably shortens the time required for antigen extraction and improves the antigen yield.

IV REAGENTS

Test Latex A	1 x 2.5 mL	Test Latex A, B, C, D, F, and G consist of blue latex
Test Latex B	1 x 2.5 mL	particles sensitized with rabbit antibody to appropriate
Test Latex C	1 x 2.5 mL	group specific antigen, suspended in buffer containing
Test Latex D	1 x 2.5 mL	0.1% sodium azide (preservative).
Test Latex F	1 x 2.5 mL	
Test Latex G	1 x 2.5 mL	
Extraction Enzyme (lyophylized)	2 x 2.1 g	When reconstituted with 12 mL of distilled water, contains 0.01% thimerosal as a preservative. The Extraction Enzyme is also used as the Negative Control.
Control +	1 x 1.0 mL	Positive Control contains a mixture of extracted antigen from streptococcal groups A, B, C, D, 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.

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"[11,12] and institutional guidelines should be followed in handling all items contaminated with blood or other body fluids. Extraction Enzyme does not always render bacteria nonviable. After use, contaminated materials must be sterilized by autoclaving.

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.

WARNING: Contact with combustible material may cause fire. Keep away from combustible material. When using do not eat or drink. Toxic if swallowed. 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.

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:

LATEX REAGENTS: Reagents are ready for use. Vials should be stored at 2-8°C. DO NOT FREEZE. Under proper conditions the reagents will retain their activity until the date shown on the bottle labels. After use, return the kit to the refrigerator, storing bottles in an upright position.

EXTRACTION ENZYME: The lyophilized **BBL™** Extraction Enzyme should be stored at 2-8°C. Under these conditions, it will retain its activity until the expiration date shown on the bottle labels. The reconstituted **BBL™** Extraction Enzyme when stored at 2-8°C will retain its activity for three months. However, the reconstituted **BBL™** Extraction Enzyme must be used within the expiration date on the vial.

CONTROL +: Ready for use; store at 2-8°C. Under these conditions activity will be retained until the date shown on the bottle label.

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

Reconstitution: Prior to use, reconstitute the lyophilized vial of **BBL™** Extraction Enzyme with 12 mL of distilled water. Store at 2-8°C. Use within 3 months of reconstitution, but prior to the expiration date on the vial.

V. SPECIMEN PREPARATION

Preparation of Cultures: 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]

VI. PROCEDURES

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

Materials Required But Not Provided: Pipette (to measure 0.4 mL), microbiological loop, Pasteur pipettes, glass or plastic tubes and water bath or heating block at 37° ± 2°C. Also required are the necessary equipment and labware used for preparation, storage and handling of serologic specimens.

Test Method: If fewer than six tests 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 a test tube appropriately and dispense 0.4 mL of **BBL™** Extraction Enzyme into the tube for each specimen to be tested.
2. Select 2-5 similar colonies with a microbiological loop and emulsify in the **BBL™** Extraction Enzyme. If the culture is mixed, avoid obvious contamination. If the colonies are small, use more than five and ensure that at least a slightly turbid suspension is obtained.
3. Incubate the tube for a total of 10 minutes at 37° ± 2°C in a water bath or heat block (see "Limitations"). After 5 min incubation remove each tube and mix by shaking for 2-3 sec, then continue the incubation. Remove and allow to cool to room temperature. The extract is now ready for use.
4. Ensure that the Test Latex has warmed to room temperature. Make sure the Test Latex suspensions are mixed by shaking and expel any Test Latex from the dropper for complete mixing.
5. Dispense 1 drop from each Test Latex to be tested onto a separate circle on the reaction card.
6. Using a Pasteur pipette add 1 drop of extract to each of the six test circles.
7. With the mixing sticks provided, spread the mixture over the entire area of the circle, using a separate stick for each.
8. 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.
9. Dispose of the reaction card in an appropriate biohazard container.

VII. INTERPRETATION OF TEST RESULTS

Positive Result: A positive result is obtained if obvious agglutination of the blue latex particles occurs within 1 min with a single Test Latex. Any weaker reaction which occurs in the presence of a substantially stronger positive reaction should be ignored.

Negative Result: A negative result is obtained if no agglutination occurs within 1 min. Faint traces of granular material may be observed in negative reactions and should be ignored.

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.

When carrying out a serological identification of streptococci, the following initial observations should be made: (1) note hemolysis, (2) note cell morphology, (3) assess colonial growth for purity and quality.

a. Rule out *Streptococcus pneumoniae*. This streptococcus is α-hemolytic, bile soluble and optochin susceptible. Other streptococci are not bile soluble and are optochin resistant.[2]

b. Aerococci are non β-hemolytic, grow in 6.5% NaCl broth and give variable reactions in the bile-esculin test and pyrrolidonyl arylamidase (PYR) test. They can be differentiated from enterococci by their arrangements in tetrads or as single cells, whereas enterococci are arranged as diplococci or short chains.[3]

c. Staphylococci and *Listeria monocytogenes* are β-hemolytic and can be distinguished from streptococci by their cellular morpholology and positive catalase reaction.[4,5]

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 five 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 positive or negative control.

POSITIVE CONTROL- Shake each Test Latex and dispense 1 drop onto a separate circle on the card. Dispense 1 drop of Control + onto each of six 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 six Test Latex suspensions should demonstrate obvious agglutination.

NEGATIVE CONTROL- Shake each Test Latex and dispense 1 drop onto a separate circle on the card. Dispense 1 drop of reconstituted **BBL™** Extraction Enzyme onto each of the six 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. No obvious agglutination should be evident for any Test Latex suspension.

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

The **BBL™ Streptocard™ Positive Control (cat # 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 (see "Availability").

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 groups B and D.[2]

Streptococcus pneumoniae share common antigenic determinant(s) with group C streptococci[6] and therefore may react with Test Latex C. *S. 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 such as that described by Lawrence *et al*.[7]

Streptococcus porcinus, which is usually associated with swine, may react with Test Latex B. Differentiation from Group B streptococcus may be based on *S. porcinus* typically giving more pronounced zones of hemolysis and a positive PYR test.[8]

A water bath or heating block should be used to ensure adequate heating during the extraction procedure. Heat transfer in an air incubator may be insufficient. However, an equilibrated beaker of water within a 37°C air incubator will suffice.

Group D streptococci may require further biochemical tests to distinguish between the enterococcal and non-enterococcal species. In such cases the bile-esculin test and 6.5% NaCl test can be used to differentiate.

The group D Test Latex may not easily react with some *S. bovis* strains. These strains may require further testing for identification.

Strains have been found which appear to have both D and G antigen.[9,10]

X. PERFORMANCE CHARACTERISTICS

A total of 555 clinical isolates and stock cultures of streptococci were tested with **BBL™ Streptocard™** Enzyme Latex Test and compared to a "commercially available latex assay." Of these 193 were group A, 146 group B, 37 group C, 31 group D, 3 group F, 70 group G. 75 of the isolates did not belong to any of these groups and gave negative reactions with all 6 Test Latex (Table 1).

Table 1

Group	No. of Strains	BBL™Streptocard™ Enzyme Latex Test	Commercial Latex Test	Correlation
A	193	193	193	100%
B	146	146	146	100%
C	37	37	37	100%
D	31	31	30	96.8%
F	3	3	3	100%
G	70	70	70	100%
Non A,B,C,D,F,G	75	75	75	100%

In a further study of 22 known group D streptococci strains also possessing Group G antigen, the **BBL™ Streptocard™** Enzyme Latex Test correctly identified all 22 strains as Group D.

In a study of the **BBL™ Streptocard™** Enzyme Latex Test with 55 non-streptococcal strains from 14 different genera, one strain of *Escherichia coli* gave a positive reaction with the group C. All other reactions were negative.

XI. AVAILABILITY

Cat. No.	Description
240950	**BBL™ Streptocard™** Enzyme Latex Kit, 50 Tests.
240951	**BBL™ Streptocard™** Acid 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.
240962	**BBL™ Streptocard™** Test Latex D, 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.
237013	**BBL™ QualiSwab™** *Enterococcus faecalis* Group D, ATCC™ 29212, one swab.
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 BD Diagnostic Systems Technical Services, toll free (800) 638-8663.

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