Clinical Laboratory Department POLICY AND PROCEDURE

POLICY NUMBER: 1086
VERSION: 4

SUBJECT: Streptocard Acid Latex Test for the rapid identification of ß-hemolytic streptococci of Lancefield types A,B,C,F and G

Principle

β-hemolytic streptococci can be differentiated into Lancefield groups based on specific carbohydrate antigens. Differentiation is necessary for clinical treatment and for epidemiological purposes. 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 latex agglutination.

Specimen

Samples for identification should be grown on a blood agar plate 16-24 hr at 33-37°C. Note the hemolytic reaction of suspect colonies. It is also necessary to perform a gram stain and catalase test to confirm the presence of Gram positive, catalase negative cocci.

Precautions

Observe established precautions against microbiological hazards throughout all procedures. All specimens must be handled according to Universal Precautions as outlined in the Infection Control Manual.

Reagents and Materials

Extraction Reagent 1 -	2.7 ml reagent, with 0.098% sodium azide as a preservative.
Extraction Reagent 2 -	2.7 mL of extraction reagent 2.
Extraction Reagent 3 -	2 bottles each containing 6.8 mL of reagent with 0.098% sodium azide as a preservative.
Test Latex	Test Latex A,B,C,D,F, and G consist of blue latex particles coated with rabbit antibody to appropriate group specific antigen, suspended in buffer with 0.098% sodium azide (preservative)
Control +	Positive control containing extracted antigen from streptococcal groups A,B,C,D,F and G.

Quality Control

Positive and negative controls are contained in the kit. Controls are to be run each day of use. Results are recorded on the Micro Quality Control log. Failure of controls to perform as expected must result in resolution of the problem before patient testing may proceed.

Specimen Preparation

1. Label one 12x75 mm test tube for each specimen to be tested.
2. Add 1 drop of Extraction Reagent 1 to each specimen tube by holding the bottle and gently squeezing.
3. Select 1-4 beta hemolytic colonies using a disposable loop or needle and suspend them in the Extraction Reagent 1. If the colonies are small, pick several well isolated colonies to be tested such that the Extraction Reagent 1 solution becomes turbid. In all cases, the colonies should be picked from an area which will afford the lowest probability of contamination with another organism.
4. Add 1 drop of Extraction Reagent 2 to each tube.
5. Mix the reaction by gently tapping the tube with a finger for 5-10 seconds.
6. Add 5 drops of Extraction Reagent 3 to each tube and mix by gently tapping the tube with a finger for 5-10 seconds.

Procedure

1. Re-suspend the test latex reagents by gently inverting the dropper bottle several times. Examine to ensure that the particles are properly suspended before use. Dispense 1 drop from each Test Latex to be tested onto a separate circle on the reaction card for each specimen, positive and negative control.
2. Using a Pasteur pipette, add 1 drop of extract to each of the test circles containing a drop of Test Latex to be tested.
3. Add 1 drop of positive control to each of the designated Test Latex test circles and 1 drop of the negative control to each of the designated Test Latex test circles on the card.
4. With the mixing sticks provided, spread the mixture over the entire area of the circle, using a separate stick for each.
5. Gently rock the card manually for up to 1 minute and observe for agglutination under normal lighting conditions. Read macroscopically; do not use magnification to aid reading.

Interpretation of Results

1. Controls: Each of the Test Latex should demonstrate obvious agglutination with the positive control. No obvious agglutination should be evident for any Test Latex suspension with the negative control.

2. Positive test: The test should be considered positive when agglutination occurs within 1 minute with one Test Latex or when one Test Latex gives a substantially stronger reaction the other four.
3. Negative test: No agglutination occurs after 1 minute.
4. Un-interpretable: If more than one Test Latex strongly agglutinates, then the possibility exists that a mixed culture of streptococcal groups is present. Subculture to re-isolate the organism for retesting.
5. Do not test or result any patients against the Test Latex for Group D, as we do not test/use any QC organism against this.

Limitations of 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. *Streptococcus pneumoniae* share common antigenic determinants with group C streptococci and therefore may react with Test Latex C. *Strep. pneumoniae* colonies are typically α-hemolytic. *Streptococcus milleri* possess A,C,F or G antigens and may therefore react with one or more of the Test Latex. Make sure that only β-hemolytic colonies are tested. False positive reactions have been known to occur with organisms from unrelated genera (E.coli, Klebsiella or Pseudomonas). These are likely to non-speciffically agglutinate all of the latex reagents. Some strains of Group D streptococci have been found to cross react with Group G antisera. Listeria monocytogenes may cross react with the Group B and G Streptococcal latex reagents. The catalase should be performed to distinguish between Listeria (catalase positive) and streptococci (catalase negative). Gram staining and motility testing may also be performed as further aids to differentiation. Some strains of Strep milleri (Strep anginosus) typically non-hemolytic possess A,C,F or G antigens and can give positive reactions with these latex reagents.

Reference:

BD BBL Streptocard Acid Latex Test Kit test package insert L010796(01) 07-2015. Becton, Dickinson and Company. Sparks, MD

Reviewed: 05/26/2017	**Next Review Date:** 05/26/2018
Revised:	August 27, 2001 4/15/04db-updated language to HDHS, 4/20/17jh changed approver to Dr.Yee, changed title to be more generic 5/20/10 jh-reformated
Written By:	Jill Hartenstein (CLINICAL LABORATORY SCIENTIST II)
Distribution:	