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Wampole ColorCard® Streptozyme®

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Agglutination Test

CLIA Complexity: Moderate
CDC Analyte Identifier: 0452

INTENDED USE

Wampole ColorCard® Streptozyme® is a color-enhanced slide test for the rapid qualitative and semi-quantitative detection of antibodies to Streptococcal extracellular antigens in serum, plasma and peripheral blood.

SUMMARY AND EXPLANATION

Wampole's ColorCard® Streptozyme® is a simple, rapid screen with titration method for detection of antibodies to the extracellular antigens of Group A Streptococcus. Such extracellular antigens may develop in rheumatic fever, streptococcal pharyngitis, pyoderma, glomerulonephritis and other related conditions.[2-3]

The serological procedure most widely used to detect Group A Streptococcus in symptomatic patients has been the anti-streptolysin O (ASO) test. Since streptolysin is only one of several Group A Streptococcus exoenzymes, the anti-streptolysin test will not detect the antibodies to other exoenzymes of Group A Streptococcus.

Wampole ColorCard® Streptozyme® will detect anti-streptolysin O, anti-streptokinase, anti-hyaluronidase, and antibodies to other streptococcal antigens in sera having an ASO titer of 166 Todd units or above, and in an additional 20% of patients with negative ASO titers. **Wampole ColorCard™ Streptozyme®** is therefore superior to the conventional ASO test for the detection of antibodies to extracellular antigens of Group A, and will detect more positive streptococcal exoenzyme antibody specimens than will any test for single antibodies.[1, 4, 5]

PRINCIPLE

The **Wampole ColorCard® Streptozyme®** Reagent consists of a color enhanced standardized suspension of aldehyde-fixed sheep cells, sensitized with Group A Streptococcus extracellular antigens such as streptolysin, streptokinase, hyaluronidase, DNase, and NADase, which will react with antibodies to these antigens to give a positive agglutination reaction. The added coloration facilitates the recognition of positive and negative reactions.

REAGENTS

For *in vitro* diagnostic use, refrigerate between 2˚ and 8˚C. DO NOT FREEZE.

1. **Wampole ColorCard® Streptozyme®** Reagent (Standardized sheep cells sensitized with Group A Streptococcus Extracellular Antigens): contains buffer and sodium azide 0.1% as preservative. Shake well before using.
2. Positive Control Serum (Human): In a buffer containing sodium azide 0.1% as preservative.
3. Negative Control Serum (Human): In a buffer containing sodium azide 0.1% as preservative.

PRECAUTIONS

This product contains dry natural rubber. Dry natural rubber is in the dropper bulb(s).

1. All serum blood components used to prepare controls have been tested by an FDA approved method for Hepatitis B surface antigen (HbsAg), HCV, and HIV antibodies and have been found to be non-reactive. The user is cautioned to handle reagents as if being capable of transmitting these diseases.

   WARNING: POTENTIAL BIOHAZARD MATERIAL. Because no test can offer complete assurance that HIV, HCV, hepatitis B virus or other infectious agents are absent, the controls should be handled at BioSafety Level 2 as for any other infectious human serum or blood in the Centers for Disease Control/National Institutes of Health Manual, "Biosafety in Microbiological and Biomedical Laboratories," 1993.

2. **WARNING:** The reagents in this kit contain sodium azide. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. Upon disposal, flush with a large volume of water to prevent azide buildup.

3. **WARNING:** The reagents in each kit are matched for proper sensitivity and maximum accuracy. For this reason, reagents from different kits must not be interchanged.

4. Prior to use, mix **Wampole ColorCard® Streptozyme®** Reagent well.

5. Use reagents and specimens at room temperature.

6. Use new pipette/stirrer for each sample. Do not touch end of stirrer used for mixing.

7. Do not touch surface of disposable card.

INDICATIONS OF INSTABILITY:

Reagent check test: The **Wampole ColorCard® Streptozyme®** reagents in this kit are stable when stored as directed. To insure potency, they are shipped by the fastest way possible and should not be subject to damage under normal transit conditions. However, as with all reagents, the **Wampole ColorCard® Streptozyme®** reagents can be damaged by extremes of temperature. The following procedure will quickly indicate whether the reagents have been subjected to improper transit conditions.

Test control sera included in the kit as described under steps 2-5 of "Testing of Samples-Serum". (Note: Do not dilute the controls 1:100 as described in Step 1 under "Testing of Samples-Serum"). When tested, the positive control serum should give a typical agglutination pattern while the negative control serum should illustrate a typical negative reaction.

If the test indicates the reagents have been damaged, return the kit and a replacement will be made immediately

SPECIMEN COLLECTION AND PREPARATION

The use of fresh or inactivated serum, plasma, or peripheral blood from the fingertip or earlobe is recommended.

Samples that cannot be tested within 24 hours of being drawn, should be frozen. Serum may be kept frozen for extended periods of time. A preservative such as sodium azide 0.1% or thimerosal 1:10,000 should be added to a specimen if it is to be mailed for testing.

When blood is used for testing, either the pipette/stirrer supplied in the kit or a heparinized capillary tube (not supplied) may be used.

MATERIALS PROVIDED

1. **Wampole ColorCard® Streptozyme®** Reagent, containing buffer and sodium azide 0.1%
2. POSITIVE CONTROL SERUM (HUMAN), containing buffer, stabilizer and sodium azide 0.1%
3. NEGATIVE CONTROL SERUM (HUMAN), containing buffer, stabilizer and sodium azide 0.1%
4. Pipettes/Stirrers
5. Disposable Cards

MATERIALS NOT PROVIDED

1. Test tubes for dilution.
2. Timer.
3. Isotonic saline (0.85% sodium chloride).
4. Pipettes or syringes with large hypodermic needles for dilution.
5. High intensity light source.

TEST PROCEDURE (QUALITATIVE)

In order to obtain accurate and reproducible results, the test procedure must be carefully followed.

SERUM OR PLASMA TESTING

1. Dilute the sample 1:100 with isotonic saline. **DO NOT DILUTE** Positive and Negative Control.

2. Using a clean pipette/stirrer, place one free falling drop of diluted patient sample (50µL) into the center of a circle. Retain stirrer for mixing (step 4).

3. Add one drop of **Wampole ColorCard® Streptozyme®** reagent.

4. Using the flat end of appropriate pipette/stirrer, spread the mixture uniformly over the entire test area.

5. Rock slide gently with rotary motion for two minutes and immediately observe for agglutination. A direct light source above the slide facilitates reading. Read results at 2 minutes. After 2.5 minutes, drying of reagents on the slide could cause a false positive result.

BLOOD (PERIPHERAL)

1. Draw blood from suitable area (fingertip, earlobe, etc.).

2. Using a clean pipette/stirrer, and without allowing blood to clot, extract and expel one drop (50µL) into a tube containing 2.5mL isotonic saline. On the basis of a 50% hematocrit, this 1:50 blood dilution is equivalent to a 1:100 serum dilution.

3. Proceed using steps 2, 3, 4 and 5 under Serum or Plasma Testing.

TEST PROCEDURE (SEMI-QUANTITATIVE)

To obtain the titer of **Wampole ColorCard® Streptozyme®** antibody, prepare the dilutions illustrated in the following table:

PREPARATION of DILUTIONS for Wampole ColorCard™ Streptozyme® TITER

0.1mL serum or plasma sample	+	9.9mL saline	=	1:100
1.0mL primary dilution	+	1.0mL saline	=	1:200
0.5mL primary dilution	+	1.5mL saline	=	1:400
0.5mL primary dilution	+	2.5mL saline	=	1:600
0.5mL primary dilution	+	3.5mL saline	=	1:800

NOTE: When blood (peripheral) is used, follow the above dilution procedure, making sure the blood is not allowed to clot, if no anticoagulant is used.

QUALITY CONTROL

It is recommended that a positive and a negative control be tested for each new test kit and as often as the needs of the lab dictate.

INTERPRETATION OF RESULTS

Qualitative Test Results

NEGATIVE No agglutination or slightly granular with no visible color change is a negative result.

POSITIVE A positive result is a visible agglutination of brown particles against a greenish background.

Quantitative Test Results

Utilizing the titration procedure, the last dilution that shows a positive agglutination on the slide is considered the Wampole ColorCard Streptozyme titer, and should be reported as the **Wampole ColorCard® Streptozyme®** titer.

When using blood, assuming a 50% hematocrit, the final blood dilution represents half the serum dilution in the above table, e.g., the 1:100 dilution of blood is equivalent to a 1:200 dilution of serum.

LIMITATIONS

It is important to note that serial titrations, performed weekly or biweekly for up to six weeks following the initial streptococcal infection, is much more significant than a single determination. The titer of positive sera should be determined using the Semi-Quantitative procedure. The progress of the disease and treatment can only be determined by sequential determination of the patient's antibody titer.

EXPECTED VALUES

Todd units are commonly used to express ASO titers. Todd units refer to the reciprocal value of the highest dilution which shows no hemolysis.[6]

It is important to note that Todd units refer to ASO and only ASO, whereas **Wampole ColorCard® Streptozyme®** detects antibodies to multiple streptococcal extracellular antigens, including Streptolysin O.[7] For this reason, it is technically incorrect to express the titer (dilutions) in Todd units.

It is possible for a patient to have a significant **Wampole ColorCard® Streptozyme®** titer and still have a negative response to ASO. Approximately 20% of patients that tested positive with **Wampole ColorCard® Streptozyme®** titers of 1:100 or higher will have negative ASO titers (less than 166 Todd units).

PERFORMANCE CHARACTERISTICS

Comparison Studies

QUALITATIVE TEST

One hundred ninety eight (198) serum and plasma specimens were examined employing the **Wampole ColorCard® Streptozyme®** test and a commercially available slide test for detection of antibodies to extracellular antigens of Group A Streptococcus. The specimens tested were a mixture of ASO positives, ASO negatives, and unknown specimens, diluted 1:100 with isotonic saline. The tests were performed according to their respective manufacturers' direction inserts. One hundred and forty six (146) of the same samples were positive by both assays, and fifty two (52) of the samples were negative by both assays.[4]

SEMI-QUANTITATIVE TEST

When forty (40) positive specimens were compared using the semi-quantitative titration test procedure, the **Wampole ColorCard® Streptozyme®** test exhibited a slightly enhanced sensitivity and readability compared with the commercial test.[4]