Strepto Group test

Latex test for identification of Streptococci of groups A, B, C, D, F and G from human specimens.

Technical sheet

IVD CE

ORDERING INFORMATION

Format	Code	Content
Kit 6 x 50 det.	REF CSI087315	Kit
Kit 1 x 50 det.	REF CSI087331	Latex A
Kit 1 x 50 det.	REF CSI087332	Latex B
Kit 1 x 50 det.	REF CSI087333	Latex C
Kit 1 x 50 det.	REF CSI087334	Latex D
Kit 1 x 50 det.	REF CSI087335	Latex F
Kit 1 x 50 det.	REF CSI087336	Latex G
Kit 1 x 50 det.	REF CSI087337	Enzymatic Extracting Reagent
-	REF CSI087338	Positive Control
Kit 3 x 50 det.	REF CSI087339	Extracting Reagents

INTRODUCTION

The classical method for streptococcal grouping, according to Lancefield, is based on extraction of soluble antigens and their identification using specific antibodies. This serological classification is commonly adopted, even if there are some human pathologies caused from streptococci without Lancefield Group specific antigens. Different extraction procedures can be used, first the Lancefield acid-heating technique as well as Fuller's formamide-heating that are suitable for all groups. Other techniques, simpler and time saving, are described from several authors. Sclavo diagnostics method utilizes a simple chemical procedure that allows to identify groups A, B, C, F and G without cross-reaction. The strains that show negative results are retested using a direct or enzymatic extraction procedure, suitable for the group D antigen. The serological reaction is revealed by using latex particles sensitized with the specific antibody, one suspension for each group.

PRINCIPLE OF METHOD

Some well isolated colonies, are mixed with chemical extraction reagents to liberate the group antigen. This antigen is spread on different circles of the testing card. Then latex sensitized with antibodies specific for each group, is added. If the correspondent antigen is present in the sample, the antigen-antibody reaction will cause a visible agglutination (clumping). If a sample shows negative reaction with latex of groups A, B, C, F, and G, select other colonies morphologically similar to the proceeding and threat them with the reagent for enzymatic extraction. Test the obtained antigen with latex for group D. A polyvalent extract of streptococci of the above mentioned groups is supplied as a control for the reliability of the latex reagents.

COMPONENTS

Ready to use reagents.

Extracting Reagent 1 - 1 x 1.5 mL
Sodium nitrite solution, ready to use.

Extracting Reagent 2 – 1 x 1.5 mL
Acetic acid solution, ready to use.

Extracting Reagent 3 – 2 x 2.5 mL
Ammonium carbonate solution, ready to use.
Contains sodium azide 0.9 g/L as preservative.

Extracting Reagent E
1x2.0 mL, lyophilized lisozyme in Tris buffer pH 8.2 + 0,2.
Contains non reactive stabilizer and *sodium azide 0,9 g/L as preservative.*
Before use, dissolve with 2.0 mL of sterile distilled water.

Latex A 1x 1.5 mL
sensitized with antibodies (from rabbit) to streptococci of group A. Ready to use.
Contains sodium azide 0.9 g/L as preservative.

Latex B 1x 1.5 mL
sensitized with antibodies (from rabbit) to streptococci of group B. Ready to use.
Contains sodium azide 0.9 g/L as preservative.

Latex C 1x 1.5 mL
sensitized with antibodies (from rabbit) to streptococci of group C. Ready to use.
Contains sodium azide 0.9 g/L as preservative.

Latex D 1x 3 mL
sensitized with antibodies (from rabbit) to streptococci of group D. Ready to use.
Contains sodium azide 0.9 g/L as preservative.

Latex F 1x 1.5 mL
sensitized with antibodies (from rabbit) to streptococci of group F. Ready to use.
Contains sodium azide 0.9 g/L as preservative.

Latex G 1x 1.5 mL
sensitized with antibodies (from rabbit) to streptococci of group G. Ready to use.
Contains sodium azide 0.9 g/L as preservative.

Positive Control – 1 x 1.0 mL
Lyophilized. Streptococci antigens of groups A, B, C, D, F and G in physiological saline. Contains non reactive stabilizer and *sodium azide 0.9 g/L as preservative.*
Before use, dissolve with 1.0 mL of sterile distilled water.

Disposable sticks (toothpicks) number 300.

Disposable black background cards number 50.

REAGENT PREPARATION

Latex reagents and extracting reagents 1, 2, and 3 and are ready to use. Bring the reagents to room temperature before use, shake the latex reagents gently to obtain an homogenous suspension of particles. After opening, the reagents are stable until the expiry date if kept as indicated in "Storage and stability".
Extracting Reagent E and Positive control are lyophilized and must be resuspended in sterile distilled water before use. If stored at 2-8 ° C and preserved from contamination , reagents are stable for 3 months .

Preservation and stability

= Storage temperature 2-8 °C

If stored closed at 2-8°C, avoiding direct light, the reagents are stable until the expiration date printed on the label. Avoid freezing and bacterial contamination.
Stability tests repeated on three different batches confirmed a total validity of 36 months if stored 2-8° C. Slight variations in composition from batch to batch do not affect test result.

REAGENTS AND EQUIPMENTS REQUIRED BUT NOT PROVIDED

- Loops for specimen collection;
- Tubes for antigen extraction;
- 15 µL pipettes;
- Adequate Containers for waste material contaminated with infectious agents.

SPECIMEN COLLECTION AND PRESERVATION

For a correct identification it is important that the colonies (which must be well isolated on blood agar) are picked up fresh . Before serological analysis, it is advisable to observe the hemolytic activity and set up a slide with Gram stain to ensure the purity of the strain to be tested .

PROCEDURE

Quality Control
Use the positive control and saline as if they were extracted from a sample. The absence of reactions (respectively positive or negative) is index of alteration of the reagents and / or controls .

Technique with Chemical Extraction

1. Distribute 30 µL (one drop) of Extracting Reagent 1 into a labelled test tube.
2. Pick up 5-6 colonies with a toothpick, being careful not to pick up part of the culture medium. Add colonies into the test tube and mix to obtain an homogeneous suspension.
3. Add 30 µL (one drop) of Extracting Reagent 2.
4. Let stand for at least 5 minutes at room temperature. Do not exceed 10 minutes. A prolonged extraction time decreases the sensitivity of the test.
5. Add 60 µL (two drops) of Extracting Reagent 3 and mix. Use within 15 minutes.

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6. Resuspend the latex reagent to be used (i.e. A, B, C, F, and/or G) by shaking the vial.
7. Holding the dropper vertically, add 1 free-falling drop of latex in one circle of the card. Repeat this operation for each latex to be used.
8. Add 15 µL of antigenic extract in each circle.
9. Using a clean toothpick, mix and spread the reaction mixture carefully. Discard the used toothpicks.
10. Tilt and rotate the card. After one minute, observe each circle for evidence of agglutination (clumping). Later agglutinations should be considered as non-specific.

If all results are negative, proceed with the technique for identification of Group D Streptococci.

Direct Technique

(This procedure is able to identify about 70% of Group D strains).

1. Transfer 30 µL (a drop) of Extracting Reagent 3 in a circle of the slide.
2. Pick up 2-3 colonies with a clean toothpick, being careful not to pick up part of the culture medium, and carefully mix them in the same circle of the slide.
3. Add a drop of Latex D.
4. Tilt the slide for 1 minute. At the end observe each circle for the presence or absence of agglutination. Later agglutinations should be considered as non-specific.

If negative results are obtained continues with enzymatic extraction technique.

Technique with Enzymatic Extraction

(This procedure is able to identify to identify more than 95% of group D strains)

1. Distribute, after reconstitution, 60 µL (two drops) of Extracting Reagent E into a labelled test tube.
2. Pick up 2-3 colonies with a clean toothpick, being careful not to pick up part of the culture medium. Insert colonies into the test tube and mix to obtain an homogeneous suspension.
3. Incubate at 37° C for 10 minutes.
4. Holding the dropper vertically, add 1 free-falling drop of Latex D in one circle of the card.
5. Add 15 µL of antigenic extract in one circle.
6. Using a clean toothpick, mix and spread the reaction mixture carefully. Discard the used toothpicks.
7. Tilt and rotate the card. After one minute, observe each circle for evidence of agglutination (clumping). Later agglutinations should be considered as non-specific.

READING OF RESULTS

Technique with Chemical Extraction

Positive (for presence of group A,B,C.F or G antigens): agglutination in the test circle with latex A,B,C.F or G respectively.

Negative (for presence of group A,B,C.F or G antigens): absence of agglutination in the test circle with latex A,B,C.F or G respectively.

Direct Technique

Positive (for presence of group D antigen): agglutination in the test circle with latex D.

Negative (for presence of group D antigen): absence of agglutination in the test circle with latex D.

Technique with Enzymatic Extraction

Positive (for presence of group D antigen): agglutination in the test circle with latex D.

Negative (for presence of group D antigen): absence of agglutination in the test circle with latex D.

Warning:

An insufficient amount of bacterial culture used can cause false negative results .

VALIDATION TEST

Sensitivity

The identification with chemical extraction technique of groups A,B,C,F and G streptococci, performed both on lyophilized collection strains and on clinical isolations, has showed a sensitivity of 98%.

The identification of group D with direct technique has showed a sensitivity of 74,3%.
The identification of group D with enzymatic extraction has showed a sensitivity of 92%.

PRECAUTIONS

Reagent disposal

Disposal must be carried out in accordance with current national, regional or European governmental provisions regarding waste.

Cautions for use of reagents

Extracting Reagents 1 and 2 are harmful. Refer to the appropriate Material Safety Data Sheet for the risk phrases and safety measures (**Risk identification**: **Reagent 1** : **GHS03 - H272, GHS06 - H301, GHS09 - H400; Reagent 2: GHS07 - H315, H319**). In addition to any risk indications related to the active components, reagents may contain inactive components such as preservatives and detergents. The total concentrations of these components are lower than the limit reported in the EC directive 1272/2008 and subsequent amendments and additions. However, It is recommended to handle reagents according to the standards of Good Laboratory Practices.

The product complies with Law Decree 8 Sept. 2000, n. 332 "Actuation of guideline 98/79/CE regarding in vitro medical diagnostic devices."

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Symbols used on labels and packaging

IVD = In vitro diagnostics medical device

REF = Catalogue number

LOT = Lot number

= Manufacturer

= Expiration date

= Storage temperature

= Instructions for use

= Warning, Read Instructions for Use

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