Strepto Group test

IVD CE

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

Instructions for Use (IFU)

ORDERING INFORMATION

Form	Code	Reagent	Composition
Kit 6 x 50 det.	REF CSI087315	Complete kit	KIT
Kit 1 x 50 det.	REF CSI087331	Latex A	n° 1 vial x 1,5 ml
Kit 1 x 50 det.	REF CSI087332	Latex B	n° 1 vial x 1,5 ml
Kit 1 x 50 det.	REF CSI087333	Latex C	n° 1 vial x 1,5 ml
Kit 1 x 50 det.	REF CSI087334	Latex D	n° 1 vial x 1,5 ml
Kit 1 x 50 det.	REF CSI087335	Latex F	n° 1 vial x 1,5 ml
Kit 1 x 50 det.	REF CSI087336	Latex G	n° 1 vial x 1,5 ml
Kit 1 x 50 det.	REF CSI087337	Extr. Reag E	n° 1 vial x 2 ml
-	REF CSI087338	Positive Control	n° 1 vial x 1 ml
Kit 1 x 50 det.	REF CSI087339	Chemical Extracting Reag.	n° 2 vials x 1,5 ml n° 2 vials x 2,5 ml

INTENDED USE

In vitro diagnostic medical device for the execution of the rapid latex agglutination test for the identification of groups A, B, C, D, F and G of Streptococcus from primary cultures obtained from samples of human origin. Test results should always be interpreted in relation to the clinical context. For professional use only.

CLINICAL SIGNIFICANCE

The classical method for streptococcal grouping, according to Lancefield, is based on extraction of soluble antigens and their identification using specific antibodies. Even if there are some species, of which the responsibility in human pathologies is ascertained, that have no group specific antigens, the Lancefield serological classification is commonly adopted. 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 abovementioned groups is supplied as a control for the reliability of the latex reagents.

Storage 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 (could reduce the validity of the kit and / or lead to incorrect results). Stability tests repeated on three different batches confirmed a total validity of 24 months if stored 2-8° C. Slight variations in composition from batch to batch do not affect test result.

COMPONENTS

All concentrations refer to ready to use reagents

Extracting Reagent 1 GHS03 GHS06 GHS09
1 x 1.5 mL, sodium nitrite solution, ready to use.
Warning: DANGER (H272; H301; H400; P220; P301+P310; P321; P330; P405; P501)
Contains sodium nitrite (CAS 7632-00-0)

Extracting Reagent 2 GHS02 GHS05
1 x 1.5 mL, acetic acid solution, ready to use.
Warning: CAUTION (H226; H314; P303+P361+P353; P305+P351+P338; P310; P321).
Contains acetic acid (CAS 64-19-7)

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
1 x 2.0 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
1x1.0 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.

Toothpicks number 300.

disposable black background cards number 50.

Legend:
Warning statements H: ***H272*** *May intensify fire; oxidiser;* ***H301*** *Toxic if swallowed;* ***H400*** *Very toxic to aquatic life;* ***H226*** *Flammable liquid and vapour;* ***H314*** *Causes severe skin burns and eye damage.*
Precautionary statements P: ***P220*** *Keep away from clothing and other combustible materials;* ***P301+P310*** *IF SWALLOWED: Immediately call a POISON CENTER/ doctor;* ***P321*** *Specific treatment (see on this label).* ***P330*** *Rinse mouth;* ***P405*** *Keep under lock and key;* ***P501*** *Dispose of contents/container in accordance with local/regional/national/international regulations;* ***P303+P361+P353*** *IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water [or shower];* ***P305+P351+P338*** *IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.* ***P310*** *Immediately call a POISON CENTER/doctor;* ***P321*** *Specific treatment (see on this label).*

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.

PRECAUTIONS and WARNINGS

1. In addition to risk statements for active components, reagents may contain non-active components such as preservatives and detergents. The total concentration of these components is below the limits set out in Regulation 1272/2008 EC and subsequent amendments and additions. However, it is recommended to handle the reagents according to the rules of good laboratory practice.
2. The results should be interpreted in the context of all available clinical and laboratory information.
3. The test cannot be used directly on clinical samples or on primary cultures in liquid medium. If a clear result is not obtained from primary cultures or mixed subcultures on solid medium, and presence of streptococci is suspected, it is recommended to set up pure subcultures of suspect colonies and then repeat the identification test with Streptogroup test.
4. From the literature is known that false positive reactions with microorganisms of unrelated genera e.g. Escherichia coli, Klebsiella or Pseudomonas are possible. These genera can agglutinate nonspecifically with all latexes. To avoid the reporting of incorrect results, the operator can exclude these microorganisms by a careful examination of the cultures developed on a plate with selective growth medium. In some streptococci the existence of antigens similar to other microorganisms has been demonstrated: in these cases, the possibility of the presence of cross-reactions cannot be eliminated. In cases where there are strains for which unambiguous classification is not possible, these must be sent to a reference laboratory for identification.
5. Identification with the STREPTOGROUP TEST is presumptive and all positive results must be confirmed by further identification tests and pure cultures serotyping.
6. If an inadequate amount of culture is used in the extraction phase, false positive or false negative results may be obtained.
7. *Listeria monocytogenes* can cross-react with anti-streptococcus latex B and G. To distinguish listeria from streptococci, perform the Catalase test: Listeria is positive catalase while streptococci are negative catalases. As a further aid in identification proceed with Gram staining.
8. Latex reagents and extracting reagents 1, 2, and 3 are ready to use. Bring to room temperature and gently shake the latex reagents to obtain a homogeneous suspension of the particles. The Extracting reagent E and the Positive Control are freeze-dried and must be dissolved in sterile distilled water before use. If stored at 2-8°C and protected from contamination, the reconstituted reagents are stable for 3 months.
9. It is important that the dropper bottle is kept upright during use and that the drop forms at the end of the spout. If the spout is wet a drop of incorrect volume may form, falsifying the test result. In this case, dry the spout before proceeding.

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10. Do not pipette by mouth, use disposable gloves and protective glasses while handling samples and performing the test. At the end wash your hands thoroughly.
11. When used in accordance with the principles of Good Laboratory Practice (GLP), with occupational hygiene operating standards, the reagents supplied do not pose any health risk.
12. The disposal of reagents must be carried out in accordance with Community provisions on waste or with the national or regional provisions in force.

Reporting of serious incidents

In the event of a serious incident in relation to the use of the device, please inform the manufacturer (via your distributor) and the competent authority of the European Union member state where the incident occurred. For other jurisdictions, reporting must be made in accordance with regulatory requirements. Reporting serious incidents helps provide more information about the safety of the diagnostic medical device.

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.

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 a 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.

SPECIMEN COLLECTION AND PRESERVATION

For more information on the collection and treatment of samples, refer to what is reported on the specialized bibliography. For the execution of the test select colonies well isolated from primary or secondary isolation cultures. 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

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 sterile toothpick, being careful not to pick up part of the culture medium. Add colonies into the test tube and mix to obtain a 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.
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 the 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 4-5 colonies with a sterile 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. Rotate 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 toothpick, being careful not to pick up part of the culture medium. Insert colonies into the test tube and mix to obtain a 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 toothpick, mix and spread the reaction mixture carefully. Discard the used sticks
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 or 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.

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

ANALITICAL PERFORMANCES

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%.

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Symbols used for IFU and Packaging	
IVD In vitro diagnostics medical device	Manufacturer
REF Catalog number	Instruction for Use
LOT Lot Number	Storage Temperature
Expiration Date	

REVISION	DATE	CHANGES
E	05/2023	MSDS Symbol Update

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