Atlas Medical

STREPTOCOCCAL GROUPING SLIDE TEST

IVD For *In-Vitro* diagnostic and professional use only

2°C 8°C Store at 2° to 8° C

CE

INTENDED USE:

ATLAS Streptococcus Latex Kit is used for qualitative detection and identification of the Lancefield group of Streptococci. Reagents are provided for groups A, B, C, D, F and G.

INTRODUCTION

ATLAS Streptococcal test uses an enzyme extraction procedure to release Carbohydrate antigen from Streptococcal cell walls.
The antigens are detected using specific antibodies to groups A, B, C, D, F and G Lancefield. These antibodies are coated on latex particles. When the antigen extract is mixed with the latex reagent, agglutination will occur. The agglutination appears as a visible clumping and can be seen macroscopically.

PRINCIPLE

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 glass slide.
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.

MATERIALS

MATERIALS PROVIDED

- **Extracting Reagent 1**: Sodium nitrite solution, ready to use.
- **Extracting Reagent 2:** Acetic acid solution, ready to use.
- **Extracting Reagent 3**: Ammonium carbonate solution, ready to use. Contains sodium azide 0.9 g/L as preservative.
- **Extracting Reagent E:** 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: sensitized with antibodies (from rabbit) to streptococci of group A. Ready to use. Contains sodium azide 0.9 g/L as preservative.**
- **Latex B**: sensitized with antibodies (from rabbit) to streptococci of group B. Ready to use. Contains sodium azide 0.9 g/L as preservative.
- **Latex C**: sensitized with antibodies (from rabbit) to streptococci of group C. Ready to use. Contains sodium azide 0.9 g/L as preservative.
- **Latex D**: sensitized with antibodies (from rabbit) to streptococci of group D. Ready to use. Contains sodium azide 0.9 g/L as preservative.
- **Latex F:** sensitized with antibodies (from rabbit) to streptococci of group F. Ready to use. Contains sodium azide 0.9 g/L as preservative.
- **Latex G:** sensitized with antibodies (from rabbit) to streptococci of group G. Ready to use. Contains sodium azide 0.9 g/L as preservative.
- **Positive Control**: 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.
- **Test slide.**
- **Stirring Sticks.**
- **Package Insert.**

NOTE: This package insert is also used for individually packed reagent.

MATERIALS NEEDED BUT NOT PROVIDED

- Water bath.
- Test tube.
- Pipettes.
- Sterile loop.

PACKAGING CONTENT

REF **8.00.13.0.0300 (5x1.5 mL Latex (A, B, C, G, F) ,1x3.0 mL Latex (D), 1x1.0 mL Positive Control, 1x 1.5 mL Extraction Reagent 1 , 1x1.5 mL Extraction Reagent 2 , 2x2.5 mL Extraction Reagent 3, 1x2 mL Extraction Reagent E, Glass Slide, plastic stirring sticks).**

STORAGE CONDITIONS

- The reagents should be stored refrigerated between 2 - 8°C avoiding direct light.
- Never Freeze or expose to elevated temperature.
- The reagent is stable until the expiry date stated on the product label. Do not use the reagents past the expiry date.

PRECAUTIONS

1. The reagents are intended *for in vitro diagnostic and professional* use only.
2. Do not pipette by mouth.
3. Always ensure an acceptable performance of the kit by performing the test on the Positive controls before using the kit.

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Reason: MoldSign Signature
Location: Moldova

4. Wear protective clothing and disposable gloves when dealing with samples and reagents. Wash hands after operations.
5. Test materials and samples should be discarded properly in a biohazard container.
6. Wash hands and the test table top with water and soap once the testing is done.
7. Test specimens may contain pathogenic organisms and must be handled with appropriate precautions.
8. When used in accordance with the principles of Good Laboratory Practice, good standards of occupational hygiene and the instructions in these Instructions for Use, the reagents supplied are not considered to present a hazard to health.
9. Do not use the kit if the kit label is not available or damaged.
10. Don't use the kit if damaged or the vials are leaking and discard the contents immediately.
11. The test should be performed at room temperature in a well let area with very good visibility.
12. Do not use the reagent if it contains particles as this may indicate reagent deterioration or contamination.
13. The Latex Suspensions and Positive Control contain 0.9g/l sodium azide . Azides can react with copper and lead used in some plumbing systems to form explosive salts. The quantities used in this kit are small; nevertheless when disposing of azide-containing materials they should be flushed away with large volumes of water.
14. In accordance with the principles of Good Laboratory Practice it is strongly recommended that extracts at any stage of testing should be treated as potentially infectious and handled with all necessary precautions.
15. Extraction Reagents 2 and 3 contain a weak acid and a mild irritant respectively. Avoid direct contact by wearing suitable protective equipment. If the material comes into contact with the skin, mucous membranes or eyes immediately wash the area by rinsing with plenty of water.

REAGENT PREPARITION

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 CONDITIONS". Extracting Reagent E and Positive control are lyophilized and must be re-suspended in sterile distilled water before use. If stored at 2-8 ° C and preserved from contamination, reagents are stable for 3 months.

SPECIMEN AND SAMPLE PREPARATION

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.

PROCEDURES

Allow all reagents and samples to reach room temperature (18-30°C) before use.

A. Technique with Chemical Extraction

1. Transfer **30 µL (one drop) of Extracting Reagent 1** into a labelled test tube.
2. Pick up 5-6 colonies with a stirring stick, 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. Transfer **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. Transfer **60 µL (two drops) of Extracting Reagent 3** and mix. Use within 15 minutes.
6. Re-suspend 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 glass slide. Repeat this operation for each latex to be used.
8. Transfer **15 µL of antigenic extract** in each circle.
9. Using a clean stirring stick, mix and spread the reaction mixture carefully. Discard the used stirring stick.
10. Tilt and rotate the glass slide. After one minute, observe each circle for evidence of agglutination (clumping). Later agglutinations should be considered as nonspecific.
    NOTE: If all results are negative, proceed with the technique for identification of Group D Streptococci.

B. 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 stirring stick, 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 nonspecific.
   NOTE: If negative results are obtained continues with enzymatic extraction technique.

C. Technique with Enzymatic Extraction

(This procedure is able 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 stirring stick, 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 glass slide.
5. Add **15 µL of antigenic extract** in one circle.
6. Using a clean stirring stick, mix and spread the reaction mixture carefully. Discard the used stirring stick.
7. Tilt and rotate the glass slide. After one minute, observe each circle for evidence of agglutination (clumping). Later agglutinations should be considered as nonspecific.

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 .

READING THE RESULT

A. Technique with Chemical Extraction

Positive: If Agglutination appears in the test circle with latex A, B, C, F or G respectively.
Negative: Fine particles appear in the test circle with latex A, B, C, F or G respectively with **no agglutination or clumping**.

B. Direct Technique

Positive: If Agglutination appears in the test circle with latex D.
Negative: Fine particles appear in the test circle with latex D with **no agglutination or clumping**.

C. Technique with Enzymatic Extraction

Positive: If Agglutination appears in the test circle with latex D.
Negative: Fine particles appear in the test circle with latex D with **no agglutination or clumping**.
NOTE: An insufficient amount of bacterial culture used can cause false negative results.

PERFORMANCE CHARACTERISTICS

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%.

REFERENCES

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PPI1415A01
Rev G (18.10.2023)

REF	Catalogue Number		Temperature limit
IVD	*In Vitro* diagnostic medical device		Caution
Σ	Contains sufficient for <n> tests and Relative size		Consult instructions for use (IFU)
LOT	Batch code		Manufacturer
	Fragile, handle with care		Use-by date
	Manufacturer fax number		Do not use if package is damaged
	Manufacturer telephone number		Date of Manufacture
	Keep away from sunlight		Keep dry
CONTROL +	Positive control	CONTROL -	Negative control

GRAM STAIN PACK

IVD **For *in -vitro* diagnostic and professional use only**

15°C 30°C **Store at Room Temperature**

INTENDED USE

Gram Stain used for differentiate between gram positive and gram-negative bacteria.

INTRODUCTION

Gram staining is used to differentiate bacterial species into two large groups (Gram-positive and Gram-negative) based on the physical properties of their cell walls.

PRINCIPLE

Gram-positive bacteria have a thick mesh-like cell wall made of peptidoglycan (50-90% of cell wall), which stains Blue while gram-negative bacteria have a thinner layer (10% of cell wall), which stains pink. Gram-negative bacteria also have an additional outer membrane which contains lipids, and is separated from the cell wall by the periplasmic space. There are four basic steps of the Gram stain, which include applying a primary stain (crystal violet) to a heat-fixed smear of a bacterial culture, followed by the addition of a trapping agent (Gram's iodine), rapid decolorization with alcohol or acetone, and *counterstaining* with safranin or basic fuchsin.

Crystal violet (CV) dissociates in aqueous solutions into CV^+ and chloride (Cl^-) ions. These ions penetrate through the cell wall and cell membrane of both gram-positive and gram-negative cells. The CV^+ ion interacts with negatively charged components of bacterial cells and stains the cells Blue.

Iodine (I^- or I_3^-) interacts with CV^+ and forms large complexes of crystal violet and iodine (CV–I) within the inner and outer layers of the cell. Iodine is often referred to as a mordant, but is a trapping agent that prevents the removal of the CV-I complex and therefore color from the cell.

When a decolorizer such as alcohol or acetone is added, it interacts with the lipids of the cell membrane. A gram-negative cell will lose its outer membrane and the lipopolysaccharide layer is left exposed. The

CV–I complexes are washed from the gram-negative cell along with the outer membrane. In contrast, a gram-positive cell becomes dehydrated from an ethanol treatment. The large CV–I complexes become trapped within the gram-positive cell due to the multilayered nature of its peptidoglycan. The decolorization step is critical and must be timed correctly; the crystal violet stain will be removed from both gram-positive and negative cells if the decolorizing agent is left on too long (a matter of seconds).

After decolorization, the gram-positive cell remains Blue. and the gram-negative cell loses its Blue. color. Counterstain, which is usually positively charged safranin or basic fuchsin, is applied last to give decolorized gram-negative bacteria a pink or red color.

MATERIALS

MATERIALS PROVIDED

- Crystal Violet.
- Gram Iodine.
- Gram Decolouriser.
- Counterstain – Safranin O.

Note: This package insert is also used for individually packed reagent.

Packaging Content

- REF **8.17.009.0400 (1x100ml Crystal Violet, 1x100ml Iodine Solution, 1x100ml Decolouriser, 1x100ml Safranin O)**
- REF **8.17.008.1000 (1x250ml Crystal Violet, 1x250ml Iodine Solution, 1x250ml Decolouriser, 1x250ml Safranin O)**
- REF **8.17.009.1000 (1x250ml Crystal Violet, 1x250ml Iodine Solution, 1x250ml Decolouriser, 1x250ml Safranin O)**
- REF **8.15.032.0250 (1x250ml Crystal Violet)**
- REF **8.15.049.0250 (1x250ml Iodine Solution)**
- REF **8.15.051.0250 (1x250ml Decolouriser)**
- REF **8.15.125.0250 (1x250ml Safranin O)**

STORAGE AND STABILITY

- Store at room temperature.
- Stain Solution is stable up to the printed expiry date.
- Keep the bottles tightly closed to prevent air oxidation.

PRECAUTIONS

- The reagent may cause eye, skin and respiratory tract irritation; so protective clothing should be worn when handling this reagent.
- The reagent is intended for in vitro diagnostic use only.
- Do not use this reagent if the label is not available or damaged.
- Test materials and samples should be discarded properly in biohazards container.
- This reagent is considered toxic, so do not drink or eat beside it.
- Wash hands and test table top with water and soap once the testing is done.

PROCEDURE

1. immerse the heat fixed smears with Crystal Violet and allow to stain for up to 1 minute.
2. Wash with tap water.
3. Flood the smear with Gram Iodine for 2 minutes.
4. Wash with tap water.
5. Decolorize the smear for few second only.
6. Wash thoroughly with tap water.
7. Counterstain with Safranin O for up to 2 minutes.
8. Wash and allow to dry.
9. Examine under microscope using oil immersion objective

RESULTS

- Gram positive organisms (Blue).
- Gram negative organisms (Red).

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PPI2112A01
Rev C (27.03.2023)

REF	Catalogue Number		Temperature limit
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LOT	Batch code		Manufacturer
	Fragile, handle with care		Use-by date
	Manufacturer fax number		Do not use if package is damaged
	Manufacturer telephone number		Date of Manufacture
	Keep away from sunlight		Keep dry
	Flammable		