99-08-300

ストレプトLA「生研」

**In Vitro Diagnostic Reagents**
**Ref. (01E) No. 0091**

## Kit for the Grouping of Hemolytic Streptococcus by Slide Latex Agglutination

# STREPT LA "SEIKEN" for 50 samples

Streptococci are defined as "catalase-negative, facultative and anaerobic cocci which show gram-positive, concatenate or pair alignments." Streptococci are classified by hemolyticity, Lancefield's group-specific polysaccharide antigen[1] (20 groups from A to V except I and J) and some biochemical properties.

No less than 95% of streptococci isolated from human infections fall into groups A, B, C, and G. Aside from some non-hemolytic strains in Group B, there are hemolytic streptococci showing $\beta$ hemolysis.

Group A streptococci (Streptococcus pyogenes) are the causative bacteria of infections such as suppurative and systemic diseases, while Group B streptococci (S. agalactiae) cause neonatal infections, meningitis, septicemia and endocarditis. In addition, Group C streptococci (S. equi, S. zooepidemicus) and Group G streptococci (popular name: S. canis) are known as causative bacteria of wound infection and endocarditis.

This kit contains reagents for slide latex agglutination, which are used to prepare latex reagents sensitized with anti-Group A, anti-Group B, anti-Group C, and anti-Group G streptococcus rabbit antibodies, and to differentiate streptococcal groups by combining the above reagents with the nitrite extraction method.[2] The characteristics of the kit are; simple and quick operation as well as superior specificity.

## KIT COMPOSITION

1. **Sensitized Latex A** 2 ml, 1 piece
   A suspension of latex sensitized with rabbit antibody against Group A streptococci, which contains sodium azide at 0.1 w/v% as a preservative.
2. **Sensitized Latex B** 2 ml, 1 piece
   A suspension of latex sensitized with rabbit antibody against Group B streptococci, which contains sodium azide at 0.1 w/v% as a preservative.
3. **Sensitized Latex C** 2 ml, 1 piece
   A suspension of latex sensitized with rabbit antibody against Group C streptococci, which contains sodium azide at 0.1 w/v% as a preservative.
4. **Sensitized Latex G** 2 ml, 1 piece
   A suspension of latex sensitized with rabbit antibody against Group G streptococci, which contains sodium azide at 0.1 w/v% as a preservative.
5. **Positive Control** 1 ml, 1 piece
   A mixed suspension of inactivated Group A, Group B, Group C, and Group G streptococci, which contains sodium azide at 0.1 w/v% as a preservative.
6. **Extracting Reagent 1** 5 ml, 1 piece
   An acetic acid solution.
7. **Extracting Reagent 2** 5 ml, 1 piece
   A sodium nitrite solution.
8. **Extracting Reagent 3** 5 ml, 1 piece
   A Tris buffer solution.
9. **Slide agglutination plate** 55 sheets
10. **Sample cup** 55 pieces
11. **Dropping pipet** 55 pieces
12. **Stirring rod** 220 pieces



## INTENDED USE

For the grouping of hemolytic streptococcus

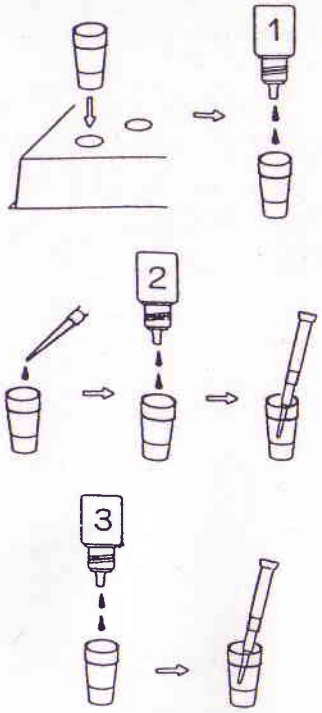
## PRINCIPLE OF MEASUREMENT

The principle of measurement is slide latex agglutination in which the latex particles sensitized with antibodies against Groups A, B, C, and G streptococci, agglutinate by reacting specifically with each streptococcal antigen group which is extracted by nitrite on the agglutination plate.

## PROCEDURE

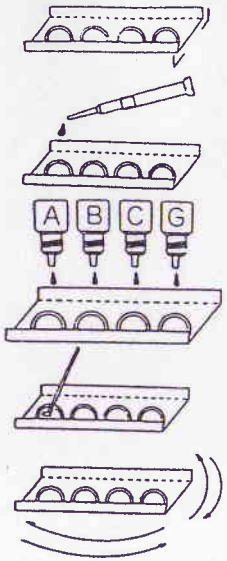
Please refer to the drawings of procedure.

1. **Sample**
   Either 100 $\mu$l of a streptococcus culture medium (Todd-Hewitt medium) isolated from the test material by a technique described in the Microbiological Test Handbook,[3] or 3 to 5 colonies of streptococci on the isolated medium (blood agar plate) should be used as a sample.
2. **Extraction of Sample**
   The reagent stand should be fixed before the following operations are carried out and should remain fixed.




1) Take a reagent stand and place a sample cup in the place on the reagent stand designed to hold the sample cup.
2) Drop two drops of extracting Reagent 1 into the sample cup.
3) Add the sample to the sample cup.
4) After adding the sample, drop two drops of extracting Reagent 2 into the sample cup.
5) Stir the contents of the sample cup with a dropping pipet and then allow it to stand for 5 minutes (leaving the dropping pipet standing in the sample cup).
6) Drop two drops of extracting Reagent 3 into the sample cup.
7) Thoroughly stir it with a dropping pipet (allowing it to stand).

### 3. Slide Latex Agglutination




1) Prepare a slide agglutination plate by bending along the dotted line.

2) Drop one drop of the extracting solution in each of the 4 circles at the slide agglutination plate.

3) Next, drop one drop each of the sensitized latexes A, B, C, and G into each of the 4 circles from left to right.

4) Spread the reaction solution over the whole area of each circle using a stirring rod. (Use a new stirring rod for each circle.)

5) Tilt the agglutination plate back and forth, right and left, for two minutes, by hand.

6) Visually determine the presence of agglutination under a suitable light immediately after completion of the reaction.

### 4. Confirmation of Reagent Effectivity

The effectiveness of agglutination and the reagent can be checked by dropping 2 drops of positive control instead of the sample into the sample cup in a similar extraction procedure and then allowing it to react with each sensitized latex.

## NOTE

1. Do not freeze this reagent. Use it after allowing it to stand at room temperature for 30 minutes or more.
2. Use the latex reagent and positive control only after thoroughly stirring them to obtain a uniform suspension. Do this immediately prior to use.
3. Drop in the reagent with the bottle standing vertically.
4. Be careful not to use the wrong cap for each reagent.

## INTERPRETATION

The interpretation of results is performed in the following manner. Cases in which apparent agglutination is noted are determined to be positive.

| Observation | Determination |
|---|---|
| When one kind of sensitized latex is positive | The latex showing a positive result shows groups of test strains. |
| When two or more kinds of sensitized latexes are positive | Determination is unclear.<br>Carry out the test after reselection of colonies because more than one group of streptococci might possibly be mixed in the sample. |
| When no agglutination is noted at all | Determination is unclear.<br>The following are possible causes:<br>(1) The volume of bacteria is extremely small.<br>(2) The bacteria are not streptococci, or are streptococci other than Groups A, B, C and G.<br>In these cases, increase the bacterial volume or check their biochemical properties. |



## PERFORMANCE

Test results of group differentiation by hemolytic streptococci:

| No. of sampled strains (Total 277 strains) | Sensitized Latex A | Sensitized Latex B | Sensitized Latex C | Sensitized Latex G |
|---|---|---|---|---|
| Group A streptococci: 59 strains | 59* | 0 | 0 | 0 |
| Group B streptococci: 137 strains | 0 | 137 | 0 | 0 |
| Group C streptococci: 16 strains | 0 | 0 | 16 | 0 |
| Group D streptococci: 10 strains | 0 | 0 | 0 | 0 |
| Group E streptococci: 1 strain | 0 | 0 | 0 | 0 |
| Group F streptococci: 3 strains | 0 | 0 | 0 | 0 |
| Group G streptococci: 48 strains | 0 | 0 | 0 | 48 |
| Group K streptococci: 1 strain | 0 | 0 | 0 | 0 |
| Group L streptococci: 1 strain | 0 | 0 | 0 | 0 |
| Group N streptococci: 1 strain | 0 | 0 | 0 | 0 |

(in-house data)

* The figures in the above table are those for strains which showed positive results in the reaction.
There were no strains which reacted with two or more sensitized latexes.

## PRECAUTIONS FOR USE OR HANDLING

1. Use these reagents for in vitro diagnosis only.
2. Disinfect the instruments after use with a suitable disinfectant, or sterilize them with high-pressure vapor, and then dispose of them.
3. Refrain from using a more than one kit of different manufacturing lot numbers together.

## STORAGE AND SHELF LIFE

Storage: 2 to 10°C

**Shelf life: Up to expiry date on the label.**

## PACKAGE

STREPT LA "SEIKEN" for 50 samples, 1 box

## REFERENCES

1. Lancefield, R. C. et al.: A serological differentiation of human and other groups of hemolytic streptococci. J. Exp. Med., **57**, 571 (1933).
2. El Kholy, A. et al.: Serological identification of group a streptococci from throat scrapings before culture, J. Clin. Microbiol. **8**, 725 (1978).
3. Supervised by the Ministry of Health and Welfare: Microbiological Test Handbook; Bacterial & fungal tests, 3rd edit, (F-2), J. Pub. Health Ass. (1987).

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