

For Research Use Only. Not for use in diagnostic procedures

Read the package insert before use

## Legionella antisera

Reagent for the typing of *Legionella pneumophila* serogroup 7 to 15

### INTENDED USE

Differentiation of *Legionella pneumophila* serogroups 7 to 15. For Research Use Only. Not for use in diagnostic procedures.

### SUMMARY AND EXPLANATION

*L. pneumophila* is known as a common causative agent of human acute pneumonia. The most frequent serogroup of human isolate is serogroup 1, although the other serogroups may cause human infection and are often isolated from various environmental sources. Because serogrouping of *L. pneumophila* must be done by anti-serogroup sera, it is important to check its serogroup.

### PRINCIPLE OF THE PROCEDURE

When tested bacterial cells are mixed with homologous antisera, visible agglutination of bacterial cells is formed by antigen-antibody reaction. The test is based on slide agglutination method.<sup>1</sup>

### CONTENTS (Materials provided)

- L. pneumophila* serogroup 7 (code 215727)
- L. pneumophila* serogroup 8 (code 215734)
- L. pneumophila* serogroup 9 (code 293572)
- L. pneumophila* serogroup 10 (code 293589)
- L. pneumophila* serogroup 11 (code 293626)
- L. pneumophila* serogroup 12 (code 293633)
- L. pneumophila* serogroup 13 (code 293640)
- L. pneumophila* serogroup 14 (code 293657)
- L. pneumophila* serogroup 15 (code 293664)

Each antiserum is rabbit antisera against each *L. pneumophila* serogroup reference strain and contains 0.08 w/v % sodium azide as a preservative.

### PRECAUTIONS

#### 1. General precautions

- 1) This product is intended for research purposes only. NOT for diagnostic purposes.
- 2) Any change or modification in the procedure described in the package insert may affect the reliability of test results.

#### 2. Precautions for specimen preparation

- 1) A non-selective medium like B-CYE agar plate should be used for culture. Use of a selective medium may be associated with inadequate production of the antigen or self-agglutination.
- 2) Test specimen should be taken from pure cultured strains. Mixing or contamination of multiple strains may produce inaccurate results.

#### 3. Precautions for handling

- 1) *Legionella* is classified as biosafety level (BSL) 2. Tests should be conducted by trained laboratory personnel in a BSL-2 facility.
- 2) Handle test samples, specimens, and containers that are in contact with the specimens as potentially infectious.
- 3) This product contains 0.08 w/v % sodium azide. In the event of

contact with skin, eyes or mouth, thoroughly flush the site with copious amounts water immediately, and seek medical treatment if any adverse reactions occur.

#### 4. Precautions for use

- 1) Avoid freezing reagents, and store according to the storage instructions described below. If frozen, this product may deteriorate and produce inaccurate results. Before use, allow the reagents to stand at room temperature (15°C~25°C) for at least 30 minutes.
- 2) Do not use reagents after the expiration date.
- 3) Do not mix reagents even if they have the same lot number.
- 4) Do not mix the reagent bottle caps.

#### 5. Precautions for disposal

- 1) Sterilize all samples, containers, and equipment used in the test according to any of the following:
  - (i) Soak in glutaraldehyde solution at a final concentration of 3.5 vol% for at least 30 minutes.
  - (ii) Soak in sodium hypochlorite solution (0.5% w/v, effective chlorine 5,000 ppm) for at least 1 hour.
  - (iii) Autoclave at 121°C for at least 20 minutes.
- 2) This product contains 0.08 w/v % sodium azide, which may react with lead or copper to form explosive heavy metal azide. When disposing of the product, flush with copious amounts of water.
- 3) Disposal of all samples and equipment should be in accordance with local or national regulations:

### STORAGE

- 1) Storage : Store at 2°C to 10°C, protected from light.
- 2) Shelf life: The shelf life is 1 year. Do not use after the expiration date indicated on the label.

### PROCEDURE

#### 1. Materials and reagents provided

- Anti-serogroup sera : 2 mL per vial
- L. pneumophila* serogroup 7
  - L. pneumophila* serogroup 8
  - L. pneumophila* serogroup 9
  - L. pneumophila* serogroup 10
  - L. pneumophila* serogroup 11
  - L. pneumophila* serogroup 12
  - L. pneumophila* serogroup 13
  - L. pneumophila* serogroup 14
  - L. pneumophila* serogroup 15

Rabbit antisera against each *Legionella pneumophila* serogroup reference strain.

Each vial contains 0.08 w/v % sodium azide as a preservative.

#### 2. Reagent, material and equipment not provided

Buffered charcoal extract agar, physiological saline, small test tubes (5 ~ 7 mL), mess pipette (5 mL), micropipette and tips (5

~ 10 µL), fluorescent lighting, bacteriological wire or loop, incubator (37°C), autoclave (121°C) or boiling water, centrifuge (bench-top centrifuge: 900 G or more; ≥3000 rpm), glass slides, and wax pencils.

### 3. Specimen preparation

#### Properties for specimen

- 1) Tested isolate must be pure cultured and bacteriological and biochemically identified as *Legionella pneumophila*.
- 2) The bacterial strain is cultured on B-CYE agar plate for 2 to 4 days at 37°C under aerobic conditions.

### 4. Other

- 1) Caution should be exercised to avoid cross contamination among sera dropped on a glass slide when specimens and sera are mixed for slide agglutination.
- 2) *L. pneumophila* serogroup 7 to 15 antisera should only be used after the sample has shown negative results with *L. pneumophila* serogroup 1 to 6 antisera.

### 5. Preparation of reagents

Use all reagents as supplied.

### 6. Procedure

Serogrouping is determined based on slide agglutination.

#### 1) Preparation of specimens

Uniformly suspend bacterial cells in physiological saline in a small test tube. Then, heat the suspension at 121°C for 15 min or at 100°C for 60 min. After heating, the suspension is centrifuged at 900 G for 20 min, supernatant is discarded, and 0.5 mL of saline is added to the sediment. The heated cells are uniformly re-suspended in physiological saline at approximately MacFarland 20, and use as the test specimen.

#### 2) Slide agglutination

Partition a glass slide into several parts with a wax pencil, and place one drop (approximately 30 µL) of group sera in each partitioned section. As a control, place 30 µL of physiological saline instead of the sera in one partitioned section as a check for self-agglutination. Use a micropipette to place 5 ~10 µL each of the test specimens above the sera and physiological saline, and mix them well with a microbiological wire or loop. Allow them to react for 1 minute while rocking the glass slide back and forth and observe for the presence of agglutination.

### INTERPRETATION OF RESULTS

Observe agglutination patterns macroscopically under light from a fluorescent lamp or other lighting system. Initially, confirm that specimens do not aggregate with the physiological saline. A specimen should be interpreted as positive if it reacts with the product and shows definite agglutination within 1 minute of mixing, while a specimen should be interpreted as negative if it shows no or weak agglutination in reaction to the product.

Results	Interpretation
Single group serum shows positive.	The group showing positive is judged as that of the specimen.
Multiple group sera show positive.	Indeterminate
All group sera show negative.	Specimens do not correspond to <i>L. pneumophila</i> serogroup 7 to 15.

### Precautions in interpretation

- 1) If the specimen and physiological saline produce agglutination, select a different colony and repeat the test.
- 2) If multiple group sera show positive, confirm that the heat treatment and pure cultures were properly performed. A positive from the multiple group sera after confirmation, may be regarded as a combined serogroup. In particular, the combined serogroup is rarely observed in *L. pneumophila* serogroup 4, 5 and 10. In this case, confirmation should be made by a quantitative agglutination test with reacted sera.

### QUALITY CONTROL

We recommend quality control with known *L. pneumophila* serogroups at first use the reagent vial.

### PACKAGE

*Legionella* Antisera in 2 mL bottles (with a dropper cap)

*L. pneumophila* serogroup 7 (code 215727)

*L. pneumophila* serogroup 8 (code 215734)

*L. pneumophila* serogroup 9 (code 293572)

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*L. pneumophila* serogroup 13 (code 293640)

*L. pneumophila* serogroup 14 (code 293657)

*L. pneumophila* serogroup 15 (code 293664)

### BIBLIOGRAPHY

1. Benson R F., et al: Classification of the genus *Legionella*, Semin. Respir. Infect. 13, 90 (1998)

### ADDITIONAL INFORMATION

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