

#### INTENDED USE

For use in the qualitative detection and specific identification of *Legionella pneumophila* serogroup 1 culture colonies from agar plates.

#### SUMMARY AND EXPLANATION

*Legionella pneumophila* serogroup 1 is the most common etiological agent of Legionnaires' disease and one of the most frequently identified *Legionella* isolates in environmental samples.<sup>1</sup>

The most available techniques used for laboratory confirmation of identifying *Legionella* isolates are the serological methods which are based on hyper-immune rabbit antisera containing antibodies directed against the somatic lipopolysaccharide or "O" antigen.<sup>2</sup> However, many *Legionella* species and serogroups have antigens in common<sup>3</sup> and cross-reactions are seen when polyclonal antibodies are used for serological identification.<sup>3</sup> The *Legionella pneumophila* serogroup 1 Latex Reagent utilizes monoclonal antibody coated polystyrene latex particles which offer highly sensitive and specific identification of *Legionella pneumophila* serogroup 1.

#### PRINCIPLE OF THE TEST

Pro-Lab *Legionella pneumophila* serogroup 1 Latex Reagent consists of a buffered suspension of latex particles coated with monoclonal antibody, specifically directed against surface antigens of *Legionella pneumophila* serogroup 1. If the test colony is *Legionella pneumophila* serogroup 1 it will agglutinate with the reagent. The degree of agglutination is then graded visually.

#### REAGENTS AND MATERIALS AVAILABLE

The Pro-Lab *Legionella pneumophila* serogroup 1 Latex Reagents are sold individually. The materials are supplied ready for use in quantities sufficient for 20 tests.

1. ***Legionella pneumophila* serogroup 1 Latex Reagent (PL.380):** Dropper bottle containing 1.1 ml of latex particles coated with IgG fraction of mouse monoclonal antibody to *Legionella pneumophila* serogroup 1. The latex particles are suspended in a buffer containing 0.098% sodium azide as preservative.
2. ***Legionella pneumophila* serogroup 1 Negative Control Latex Reagent (PL.381):** Dropper bottle containing 0.7 ml of latex particles coated with IgG fraction (normal) of monoclonal antibody. The latex particles are suspended in a buffer containing 0.098% sodium azide as a preservative.
3. ***Legionella pneumophila* serogroup 1 Positive Control Reagent (PL.382):** Dropper bottle containing 0.7 ml of control antigen of *Legionella pneumophila* serogroup 1 grown on artificial medium and killed by formalin. The control antigen is suspended in a buffer containing 0.095% sodium azide as a preservative.

#### MATERIALS REQUIRED BUT NOT PROVIDED

1. Biological safety cabinet
2. Bunsen burner
3. Inoculating loop
4. Test tubes
5. Phosphate Buffered Saline (PBS, pH 7.4)
6. Test cards with circled areas for mixing reagents and test samples
7. Mixing sticks

#### STABILITY AND STORAGE

All components should be stored at 2-8°C. **Do not freeze.** Reagents stored under these conditions will be stable until the expiry date shown on product label.

#### PRECAUTIONS

1. Reagents are for *in vitro* diagnostic use only.
2. Do not use the reagents after the expiration date shown on the product label.
3. The reagents contain a small amount of sodium azide as a preservative. Sodium azide can react explosively with lead or copper plumbing if allowed to accumulate. Although the amount of sodium azide in the reagents is minimal, large quantities of water should be used if the reagents are flushed down the sink.
4. Universal precautions should be taken in handling, processing and discarding all clinical specimens. All test materials should be considered potentially infectious during and after use and should be handled and disposed of appropriately.
5. Do not use the Latex Reagent if autoagglutination is visible. Autoagglutination indicates that contamination or deterioration has occurred.
6. The procedures, storage conditions, precautions and limitations specified in these directions must be adhered to in order to obtain valid results.
7. The reagents contain materials of animal origin and should be handled as a potential carrier and transmitter of disease.

#### PREPARATION OF CULTURES

For best results, it is recommended that fresh cultures be used.

#### TEST PROCEDURE

1. Allow specimens and reagents to reach room temperature before use.
2. Pick as many suspected colonies as possible from the Buffered Charcoal Yeast Extract medium and suspend the colonies in about 1 ml of PBS (pH 7.4). Suspected colonies refer to ones showing typical morphology and no growth on blood agar. Ideally, the suspension should have a turbidity of approximately 10<sup>8</sup> CFU per ml (McFarland Standard 0.5). However, as little as two colonies in 1.0 ml of PBS is sufficient.
3. Suspend the Latex Agglutination Reagents by gentle agitation.
4. Add 1 drop of the colony suspension to a test circle.
5. Add 1 drop of the *Legionella pneumophila* serogroup 1 Latex Reagent to the test circle.
6. Using a mixing stick stir the contents of the test circle together and then gently rock the card for 2 minutes.
7. After 2 minutes examine the test circle for agglutination. If present, please grade as outlined below.

Definition of agglutination grading:

- 0 = Identical to negative control, homogeneous suspension of PBS and latex reagent with no agglutination.
  - 1+ = Fine granulation with a turbid background.
  - 2+ = Small clumps with a turbid background.
  - 3+ = Medium clumps with a clear background.
  - 4+ = Large clumps with a clear background.
8. If agglutination is present, test the colony suspension with the Negative Control Latex Reagent using the same procedure outlined in steps 4 to 7 above.

#### QUALITY CONTROL

The *Legionella pneumophila* serogroup 1 Latex Reagent must react with the Positive Control Reagent to give a 3+ to 4+ reaction. The Negative Control Latex Reagent must not agglutinate with the Positive Control Reagent. Otherwise, the test is considered invalid.

#### INTERPRETATION OF RESULTS

Any test that is graded 1+ to 4+ with the *Legionella pneumophila* serogroup 1 Latex Reagent is considered positive, providing that the Negative Control Latex Reagent is negative.

#### LIMITATIONS OF THE PROCEDURE

1. The latex agglutination test is a presumptive diagnostic. Confirmation by biochemical tests should be done whenever possible.
2. A negative latex agglutination test does not mean the culture is not a *Legionella* species. It only indicates that the culture is not *Legionella pneumophila* serogroup 1.

#### PERFORMANCE CHARACTERISTICS

An evaluation of the Pro-Lab Diagnostics *Legionella pneumophila* serogroup 1 Latex Agglutination test performed at the Public Health Laboratory showed that the product had 100% specificity and sensitivity when tested against a panel of strains of *Legionella pneumophila* (14 *L. pneumophila* serogroup 1 and 36 strains of other serotypes).

#### REFERENCES

1. Reingold, A.L., Thomason, B.M., Brake, B.J., Thacker, L., Wilkinson, H.W., Kuritsky, J.N. 1984. *Legionella pneumophila* in the United States: The Distribution of Serogroup and Species Causing Human Illness. *J. Infect. Disease.* 149:819.
2. Wilkinson, H.W. 1988. Legionellosis, P. 320-332. In A. Balows, W.J. Hausler, Jr., M. Ohashi, and A. Turano (ed.), *Laboratory diagnosis of infection diseases, Principles and practice*, Vol.1. Springer-Verlag, New York.
3. Plakaytis, B.B., G.M. Carbone, C.P. Pau, and Wilkinson, H. W. 1987. Purified 60-Kilodalton *Legionella* Protein with *Legionella* -Specific and Non-specific epitopes. *J. Clin. Microbiol.* 25:2080-2084.

	= Use by
	= Lot number
	= Catalogue number
	= Manufacturer
	= Authorized Representative in the European Community
	= Contains sufficient for <n> tests
	= In vitro diagnostic medical device
	= Temperature limitation
	= Consult instructions for use

