

INTENDED USE

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

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.¹

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.² However, many *legionella* species and serogroups have antigens in common³, cross-reactions are seen when polyclonal antibodies are used for serological identification³. The Prolex™ *Legionella pneumophila* serogroup 1 kit agglutination system utilizes monoclonal antibody coated latex particles which offer highly sensitive and specific identification of *Legionella pneumophila* serogroup 1.

PRINCIPLE OF THE TEST

PRO-LAB *Legionella pneumophila* serogroup 1 agglutination system consists of buffered suspension of latex particles coated with monoclonal antibody, the antibody is specifically directed against surface antigens on *Legionella pneumophila* serogroup 1. When one drop of a suspension of suspected *Legionella* colonies is mixed with a drop of latex reagent and the organism is *Legionella pneumophila* serogroup 1, it will bind through its single reactive site to the sensitized latex. The mixture will cause an agglutination that is visible and the resultant clumping is graded visually.

REAGENTS AND MATERIALS AVAILABLE

1. Latex particles coated with IgG fraction of mouse monoclonal antibody to *Legionella pneumophila* serogroup 1. The latex reagent is packaged 1.1 mL per bottle.
Available as Catalogue Number: PL 380
2. Negative control latex particles are coated with IgG fraction (normal) of monoclonal antibody and packaged 0.7 mL per bottle.
Available as Catalogue Number: PL 381
3. Control antigen of *Legionella pneumophila* serogroup 1 is grown on artificial medium and killed by formalin. The control antigen is packaged 0.7 mL per bottle.
Available as Catalogue Number: PL 382
4. Test cards with circled areas for mixing reagents and test samples.
5. Mixing sticks.

STORAGE

Latex reagents and control antigens are stored at 2°-8° C. Do not freeze. The PRO-LAB *Legionella pneumophila* serogroup 1 Latex Agglutination Kit has a shelf life of 18 months from the date of manufacture when stored in the above conditions and can be used up to the date of expiry shown on the product label.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Biological safety cabinet.
2. Bunsen burner.
3. Buffered Charcoal Yeast Extract media.
4. Inoculating loop.
5. Test tubes.
6. Phosphate Buffered Saline (PBS, pH 7.4).

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 10⁸ CFU per ml. 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 cell suspension with 1 drop of the latex reagent and 1 drop of the Negative Control onto the circled areas of the card provided.
5. Mix each circled area with a new mixing stick and gently rock card for 2 minutes.
6. Read visually by examining the degree of agglutination and grade the agglutination.

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 visible groupings with a turbid background.
- 3+ = Medium clumps with a clear background.
- 4+ = Large clumps with a clear background.

INTERPRETATION OF RESULTS

Any test that is graded 1+ to 4+ with a latex reagent is considered positive, providing that the negative control is shown to be negative.

QUALITY CONTROL


The latex reagent must agglutinate the positive control at a 3+ to 4+ clumping. The negative control latex reagent must not agglutinate the control antigen. Otherwise, the test is considered invalid.

LIMITATIONS

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.











PRECAUTIONS

1. Reagents are for *in vitro* diagnostic use only.
2. As with all clinical specimens and materials, precautions should be taken against the danger of microbiological hazards. Thus, handle all materials in a manner conforming to Good Laboratory Practices.

3. Do not use the Latex Reagent if autoagglutination is visible. Autoagglutination indicates that contamination or deterioration has occurred.
4. For best results, it is recommended that fresh cultures be used. Older cultures may be mucoid and thus, a smooth suspension should be made. This may be accomplished by vortex or other suitable method.
5. The reagents contain sodium azide as a preservative.  Sodium azide can react with lead and copper and the resultant salts have explosive properties. Large volumes of water should be used to dispose of used reagents. Furthermore, sodium azide is a skin irritant. Avoid skin contact with the reagents. Do not mix with acid as this may result in the formation of hydrazoic acid, an extremely toxic gas.

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. Plikaytis, B.B., G.M. Carlone, 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
	= Attention, see instructions for use
	= 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