Technical Bulletin 138

MICROGEN BIOPRODUCTS

Identification of *Legionella pneumophilia* using Microgen Bioproducts® Legionella

What is Legionnaires' Disease?

Legionnaires' disease is a form of lung disease (pneumonia) caused by *Legionella* bacteria. The bacterium is found in freshwater environments but can also contaminate human-made water systems like showerheads and sink faucets, hot water tanks and heaters, hot tubs, plumbing systems, cooling towers and decorative water features[1]. Humans become infected when they breathe in aerosolized droplets of contaminated water. Individuals with underlying conditions that cause swallowing difficulties can become infected by aspiration of contaminated drinking water[1]. Inhalation of water contaminated with *Legionella* can give rise to respiratory symptoms including cough, shortness of breath, fever, muscle aches and headaches. In vulnerable groups such as the elderly, infirm and young children or in smokers the infection can cause severe pneumonia requiring hospitalisation. Around 1 in 10 people who get Legionnaires' disease will die from the infection[1].

Microgen Bioproducts® Legionella Latex

Microgen Bioproducts® Legionella is a latex agglutination test intended for confirmatory identification of *Legionella pneumophila* and commonly isolated *Legionella* species grown on selective media. The test is suitable for organisms derived from patients with suspected Legionella pneumonia or from environmental sources. Microgen Bioproducts® Legionella allows the separate identification of *L. pneumophila* Serogroup 1 and Serogroups 2-15* and commonly isolated *Legionella* species.

[1] www.cdc.gov/legionella

*__NOTE:__ *L. pneumophila* serogroup 16 is now mentioned in the Manual of Clinical Microbiology. Serogroup 16 should cross-react with serogroup 6, so theoretically the Microgen 2-15 latex reagent should also agglutinate with serogroup 16 isolates.

Principle of the Test

For Test Reagent 1, latex particles are coated with polyclonal rabbit antibodies raised against *L. pneumophila* Serogroup 1. For Test Reagent 2-15, latex particles are coated with polyclonal rabbit antibodies raised against serogroups 2-15. For Test Reagent Species, latex particles are coated with polyclonal rabbit antibodies raised against 10 commonly isolated *Legionella* species (see table below).

When these latex particles are mixed with a suspension containing the appropriate *Legionella* bacteria or heat killed antigens from the relevant *Legionella* bacteria, an immunochemical reaction takes place causing the latex particles to agglutinate into aggregates which are easily visible to the naked eye. The latex reagent coated with serogroup 1 antibodies will only agglutinate in the presence of serogroup 1 antigens.

The latex reagent coated with serogroup 2-15 antibodies will only agglutinate in the presence of antigens from any one of the serogroups 2-15.

The latex reagent coated with *Legionella* species antibodies will agglutinate with the 10 relevant *Legionella* species (see table below). Reactions of Test Reagent 1 with serogroup 1 antigens are generally stronger and faster than with those between Test Reagent 2-15 and serogroup 2-15 antigens.

This is because of the dilution effect of blending 15 different antisera during manufacture of Test Reagent 2-15. The *Legionella* species latex reacts in a similar manner to the *Legionella pneumophila* serogroup 1 latex.

Legionella Species (10 Target Species)
L. micdadei
L. bozemanii 1
L. bozemanii 2
L. dumoffi
L. longbeachae 1
L. longbeachae 2
L. jordanis
L. gormanii
L. anisa
L. feelei

Quality Control

The following controls should be performed each time the kit is used:

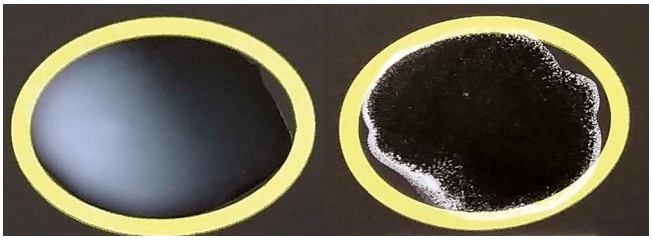
1) Reagent Control

Gently mix Test Reagent 1 (M45a) and add one drop to a well on an agglutination slide. Add one drop of saline solution (M40) to the drop of latex reagent. Mix with a mixing stick, spreading the liquid over the entire area of the well. Rock the slide gently for 2 minutes and observe for agglutination. If any agglutination is seen, at least one of the reagents is contaminated and a fresh kit should be used. Repeat this process using Test Reagent 2-15 (M45b) and Test Reagent Species (M45d). Again, no agglutination should be seen. If agglutination occurs with any of the three latex reagents, a fresh kit should be used.

2) Positive Control

Add one drop of positive control (M45c) to one well on the agglutination slide. Gently mix Test Reagent 1 (M45a) and add 1 drop to the same well. Do not allow the dropper to touch the positive control. Mix with a mixing stick, rock the slide gently for 2 minutes and observe for agglutination. A positive result, indicated by agglutination, should be seen. Repeat this process using Test Reagent 2-15 (M45b) and Test Reagent Species (M45d). Again, a positive result should be seen. If agglutination is not seen with any of the three latex reagents, a fresh kit should be used.

NOTE: The reaction strength with the positive control may not be the same for Test Reagent 1 as with Test Reagent 2-15 and Test Reagent Species. (see PRINCIPLE OF THE TEST page 1).



Reagent Control Positive Control

Interpretation

Positive Result

A positive result is indicated by the visible aggregation of the latex particles. This will normally occur within a few seconds of mixing, however the time is dependent on exact strength.

Test Reagent 1	Test Reagent 2-15	Test Reagent Species	Interpretation
+	–	–	*L. pneumophila* 1 present
–	+	–	*L. pneumophila* 2 -15 present
–	–	+	*Legionella* species present
–	–	–	No *Legionella* presents
+	+	+	*Possible nonspecific agglutination, inconclusive results
+	+	–	
+	–	+	
–	+	+	

*A non-specific agglutination pattern does not preclude the presence of *Legionella* bacteria but results have to be interpreted as inconclusive.

Negative Result

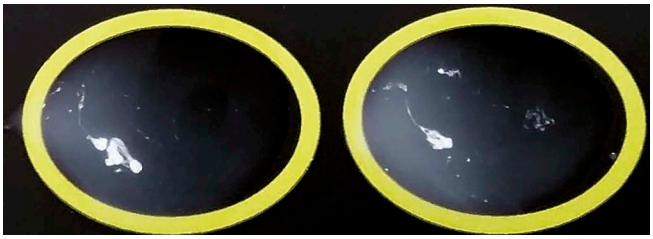
Indicated by a milky appearance without any visible aggregation of latex particles. Faint traces of granularity may be detected in negative patterns, depending on the visual acuity of the operator, these should be disregarded.

False Negative Result

False negative results can occur if an insufficient amount of culture is used for the extraction. Media from different manufacturers may elicit slow antigen expression. Users should test their media with known strains to ensure the test performs as intended before testing unknown isolates.

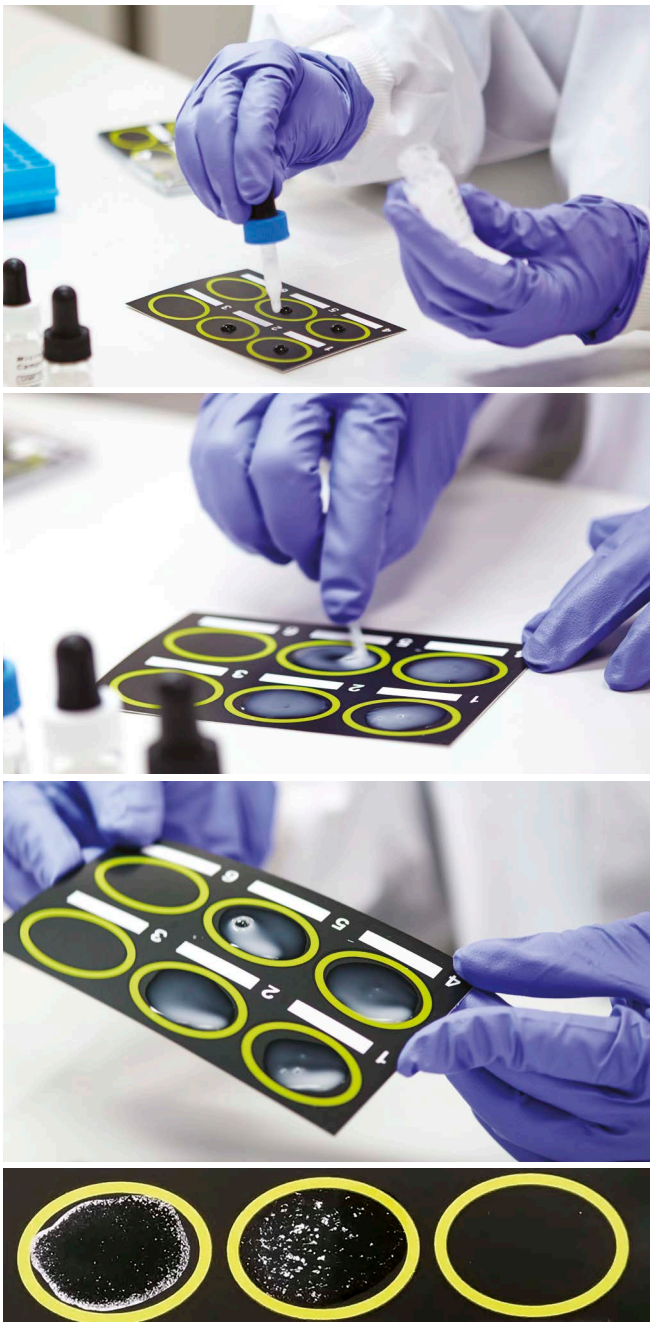
False Positive Result

Stringy reactions on the slide (often observed with a milky background below) may not be true positive reactions and further tests are required.



Microgen Bioproducts® Legionella Latex – Methods for Use

Microgen Bioproducts® latex agglutination kits are rapid slide agglutination tests for the presumptive identification of target bacteria from solid selective media.



Assay Procedure

Method for identification from selective solid media.

1) Place a disposable slide on the work bench.

2) Add 1 drop of saline to each of three wells on the disposable slide.

3) Using a mixing stick or inoculating loop, emulsify 3-4 suspect colonies in each drop of saline to produce a heavy smooth suspension, spreading the liquid over the entire surface of the well.

4) If the suspension remains smooth, proceed to step 7. If the suspension is “stringy” or “granular” (often as a result of old and/or mucoid cultures), proceed as follows:

5) Dispense 0.5mL 0.85% isotonic saline into a glass tube. Prepare a homogeneous turbid suspension of organisms taken from the selective agar plate.

6) Boil the suspension for 5 minutes. Allow to cool to room temperature. Place 30µL boiled suspension on to each of three wells of an agglutination slide.

7) Gently mix the latex reagent (M45a, M45b and M45d) to ensure a homogeneous suspension.

8) Add one drop of Test Reagent 1 to one of the bacterial suspensions, one drop of Test Reagent 2-15 to the second suspension and one drop of Test Reagent Species to the third suspension. Do not allow the reagent dropper to touch the suspension.

9) Mix the reagent and suspension using a new mixing stick for each combination. Spread the liquid over the entire area of the well.

10) Discard the used mixing sticks and slides into a suitable disinfectant.

11) Rock the slide gently for 2 minutes and observe for agglutination.

12) A positive reaction is indicated by the visible aggregation of the latex particles.

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