

LEGIONELLA LATEX TEST KIT PLUS

For professional *in Vitro* diagnostic use onlyFor the rapid confirmatory identification of *Legionella pneumophila* 01, *Legionella pneumophila* 2-15, *Legionella* spp

INTENDED USE

Legionella Latex Test Kit Plus 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 pneumonia or from environmental sources. Legionella Latex Test Kit Plus allows the separate identification of *L. pneumophila* Serogroup 1 and Serogroups 2-15 and *Legionella* spp.

PRINCIPLE OF THE TEST

The test latexes used for Legionella Latex Test Kit Plus are coated with immunoglobulins which have been specially processed to minimise cross reactions with potentially cross reacting organisms.

Reagent test L1 contains latex particles coated with polyclonal rabbit antibodies raised against *L. pneumophila* Serogroup 1. Reagent test L2-15 contains latex particles coated with polyclonal rabbit antibodies raised against serogroups 2-15. Reagent test LSPP contains latex particles coated with polyclonal rabbit antibodies raised against 10 commonly isolated *Legionella* species: *L. micdadei*, *L. bozemanii* 1 & 2, *L. dumoffii*, *L. longbeachae* 1 & 2, *L. jordanis*, *L. gormanii*, *L. anisa* and *L. feelei*.

When the 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.

REAGENTS AND MATERIALS PROVIDED

REAG TEST L1 – 2,5 mL; latex particles coated with polyclonal rabbit antibodies against *L. pneumophila* Serogroup 1. Contains 0.099% sodium azide. (**green** cap)

REAG TEST L2-15 : 2,5 mL; latex particles coated with polyclonal rabbit antibodies against *L. pneumophila* Serogroup serogroups 2-15. Contains 0.099% sodium azide. (**white** cap)

REAG TEST LSPP : 2,5 mL; latex particles coated with polyclonal rabbit antibodies against 10 commonly isolated Legionella species *L. micdadei*, *L. bozemanii* 1 & 2, *L. dumoffii*, *L. longbeachae* 1 & 2, *L. jordanis*, *L. gormanii*, *L. anisa* and *L. feelei*. Contains 0.099% sodium azide. (**black** cap)

REAG CONTROL: Control Latex Reagent: 2.5mL - Latex particles coated with non-specific rabbit immunoglobulins. Preserved with 0.099% sodium azide. (**red** cap)

CONTROL + : 1 mL; **POSITIVE CONTROL**: suspension of inactivated *Legionella* antigens reactive with Reagents L1, L2-15 and LSPP. Contains 0.099% sodium azide. (**red** cap)

SAMPLE DILUENT : 1 x 10 mL; 0.9% Saline containing 0.095% sodium azide (**vial**)

Disposable agglutination cards : 35 cards, each with 6 black agglutination areas

Mixing Sticks : 200 disposable mixing sticks

Plastic dropper - 1

Instruction for use

MATERIALS REQUIRED BUT NOT SUPPLIED

- Bacteriological loops
- Legionella selective medium
- Glass tubes for boiling
- Saline solution
- Incubator
- Timer

WARNINGS AND PRECAUTIONS

Safety:

- The reagents supplied in this kit are for *in vitro* diagnostic use only.
- The kit is intended for professional use only.
- Sodium azide, which is used as a preservative in the kit reagents, can react with lead or copper plumbing to form potentially explosive metal azides. Dispose by flushing with a large volume of water to prevent azide build-up.
- Appropriate precautions should be taken when handling or disposing of potential pathogens. Decontamination of infectious material can be achieved with sodium hypochlorite at a final concentration of 3% for 30 minutes. Liquid waste containing acid must be neutralised before treatment.
- The positive control has been inactivated during the manufacturing process. However, it should be handled as though potentially infectious.

Procedural:

- Legionella Latex Test Kit Plus should be used according to the kit instructions.
- Allow all reagents to reach room temperature before use.
- Do not dilute any of the kit reagents
- Do not intermix reagents from different batches of kits.
- Do not freeze any of the kit reagents
- Do not allow the latex reagent dropper to touch the positive control or bacterial samples.
- Be careful only to record agglutination. Reactions that are "curdy" or "stringy" may not be true agglutination.
- Ensure the agglutination card is clean and dry prior to use.

STORAGE AND SHELF LIFE

Legionella Latex Test Kit Plus must be stored at 2-8°C when not in use. The kit must not be used after the expiry date printed on the label.

SPECIMENS

The sample consists of colonies isolated on a selective agar medium (e.g. Legionella BGYE Selective Agar, REF 549995).



TECHNIQUE

Quality Control:

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

1. Reagent Control: Gently mix latex reagents (**REAG TEST L1**, **REAG TEST L2-15**, **REAG TEST LSPP** and **REAG CONTROL**) and add one drop of each reagent to separate wells on the agglutination card. Add one drop (50 µL) of **SAMPLE DILUENT** to each drop of latex reagent. Using a different mixing stick for each well, mix the latex reagent and saline thoroughly, spreading the liquid over the entire area of the well. Rock the card gently for 2 minutes and observe for agglutination. If agglutination will occur the reagent is contaminated and a fresh kit should be used.

2. Positive Control: Add one drop of **CONTROL+** to one well on the agglutination card. Gently mix **REAG TEST L1** 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 card gently for 2 minutes and observe for agglutination. A positive result, indicated by agglutination, should be seen. Repeat this process using **REAG TEST 2-15**, and **REAG TEST LSPP**. A positive result should be seen. If agglutination is not seen with any of the three latex reagents, a fresh kit should be used. Repeat this process using **REAG CONTROL**; there must be no reaction.

NOTE:

Reactions of REAG TEST L1 with serogroup 1 antigens are generally stronger and faster than with those between REAG TEST L2-15 and serogroup 2-15 antigens. This is because of the dilution effect of blending 15 different antisera during manufacture of Test Reagent 2-15. REAG TEST LSPP reacts in a similar manner to the REAG TEST L1.

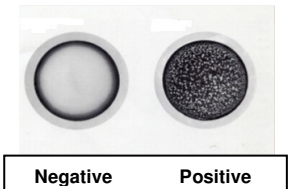
Test Procedure

1. Isolate *Legionella* colonies on an appropriate selective medium.
2. Dispense 1 drop (50 µL) of **SAMPLE DILUENT** on to each of four wells of the agglutination card.
3. Using an inoculating loop, remove 3-4 *Legionella*-like colonies from the selective agar plate and make a thick even smear on the card alongside each drop of saline.
4. By means of a mixing stick, mix the colonies with the **SAMPLE DILUENT** and emulsify to form a smooth heavy suspension, spreading the liquid over the entire surface of the well.
5. If the suspension remains smooth, proceed to point 6. If the suspension is "stringy" or "granular" (often as a result of old and/or mucoid cultures), proceed as follows:
Dispense 0.5mL 0.85% isotonic saline into a glass tube. Prepare a homogeneous turbid suspension of organisms taken from the selective agar plate. 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 card.
6. Gently mix each test latex reagents to ensure a homogeneous suspension
7. Add one drop of **REAG TEST L1** to one of the bacterial suspensions, one drop of **REAG TEST L2-15** to the second suspension, one drop of **REAG TEST LSPP** to the third suspension and one drop of **REAG CONTROL** to the fourth. Do not allow the reagent dropper to touch the suspension.
8. Mix the reagent and suspension using a new mixing stick for each combination. Spread the liquid over the entire area of the well.
9. Rock the card gently for 2 minutes and observe for agglutination.
10. Discard the used mixing sticks and cards into a suitable disinfectant.

INTERPRETATION

An agglutination reaction is indicated by visible aggregation of the latex particles. Legionella Rapid Latex Test Kit results should be interpreted as follows:

| REAG TEST L1 | REAG TEST L2-15 | REAG TEST LSPP | REAG CONTROL | Interpretation |
|--------------|-----------------|----------------|--------------|---|
| + | - | - | - | <i>L. pneumophila</i> 1 present |
| - | + | - | - | <i>L. pneumophila</i> 2-15 present |
| - | - | + | - | <i>Legionella</i> species present |
| - | - | - | - | No <i>Legionella</i> present |
| + | + | + | - | Possible non specific agglutination, inconclusive result* |
| + | + | - | - | Possible non specific agglutination, inconclusive result* |
| + | - | + | - | Possible non specific agglutination, inconclusive result* |
| - | + | + | - | Possible non-specific agglutination. Inconclusive result* |
| - 0 + | - 0 + | - 0 + | + | Result not interpretable. The strain tested cause autoagglutination |



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

LIMITATIONS OF USE

1. Results should be interpreted in the context of all available clinical and laboratory information.
2. Stringy reactions on the card may not be true positive reactions and further tests are required.
3. Old and/or mucoid cultures may not give a smooth suspension in saline and may give atypical agglutination. These should be pre-treated by boiling as described in the method above.
4. Legionella Latex Test Kit Plus is intended for the identification of *L. pneumophila* serotypes 1-15 and detection of a range of 10 *Legionella* species following culture on selective agar plates. Colonies giving positive results should be confirmed as *Legionella* spp. by their inability to grow on Legionella selective culture media deficient in cysteine (e.g. Legionella Agar w/o Cysteine REF 549943)

PERFORMANCE CHARACTERISTICS

Legionella Latex Test Kit Plus has been evaluated in comparison with a well-established, commercially available latex agglutination test for *L. pneumophila*. 128 isolates of *L. pneumophila*, non-*pneumophil*, *Legionella* spp and potentially cross-reacting bacteria were tested in both products.



| | | Legionella Latex Test Kit Plus | | Total |
|-----------------------|-----|--------------------------------|-----|-------|
| | | +ve | -ve | |
| Commercial Latex test | +ve | 50* | 0 | 50 |
| | -ve | 0 | 78 | 78 |
| Total | | 50 | 78 | 128 |

Sensitivity: 50/50 = 100%; Specificity: 78/78 = 100% ; Accuracy: 128/128 = 100%

* Of the 50 isolates in this group, 12 were cross reactants in both tests. These were isolates of *S. aureus* (4), *C. diversus* (1), *A. baimannii* (2), *P. stuartii* (1), *B. cereus* (2), *K. pneumoniae* (1), *Streptococcus spp.* (1). However all of the above either do not grow or show very atypical morphology, when cultured on Legionella selective media. In the case of *B. cereus*, agglutination was atypical (stringy)

70 isolates of *L. pneumophila* and *Legionella spp* were tested in both Legionella Latex Test Kit Plus and the comparison latex kit to differentiate between Serogroup 1 and Serogroups 2-15.

| | | Legionella Latex Test Kit Plus | | | Total |
|-----------------------|-------------------|--------------------------------|-----------------|-------------------|-------|
| | | SG1 Positive | SG2-15 Positive | L. pneu. Negative | |
| Commercial Latex Test | SG1 Positive | 8 | 0 | 0 | 8 |
| | SG2-15 Positive | 0 | 30 | 0 | 30 |
| | L. pneu. negative | 0 | 0 | 32 | 32 |
| Total | | 8 | 30 | 32 | 70 |

No isolates of *L. pneumophila* serogroup 1 cross-reacted with Reagent L2-15 and, similarly, no isolates of *L. pneumophila* serogroups 2-15 cross-reacted with Reagent L. All non-*pneumophila* *Legionella spp* isolates were non-reactive with Reagent L1 and Reagent L2-15 in Legionella Latex Test Kit Plus except ***L. longbeachae* 1&2 that produces atypical agglutination with Reagents L1 and L2-15.**

REAG TEST LSPP has been tested against all *Legionella pneumophila* serogroups with no cross-reactivity detected.

Specificity testing of the REAG TEST LSPP indicates that isolates of *Legionella sainthelensi* sgp 1, *erythra*, *hacklaie* sgp 1 and *tucsonensis* can also produce positive results.

REPRODUCIBILITY

Intra-batch reproducibility was established by testing sensitivity and specificity of 1 batch of product against serial dilutions of reference and kit control antigens and a panel of 47 bacterial samples. Different operators carried out tests on 3 separate occasions. End-point titres obtained with reference/control antigens and qualitative results with the panel were identical in the three assays.

Inter-batch reproducibility was examined by testing sensitivity and specificity of 3 batches of product against serial dilutions of reference and kit control antigens, and a panel of 47 bacterial samples. Between the 3 batches, no significant differences in end-point titres were seen and qualitative results with the panel correlated 100%.

| | | | | | | | |
|------------------------------|------------------------------------|------------------------|------------------|-------------------|---------------------------|---------------------|-------------|
| IVD | In Vitro Diagnostic Medical Device | Temperature limitation | LOT | Batch code (EXXX) | Manufacturer | Keep dry | Non-sterile |
| Consult Instructions for use | Use by (year/month) | REF | Catalogue number | Do not reuse | Fragile, handle with care | Keep away from heat | |

CONTENT (50 tests)

| | |
|---------------------------------------|-------------------------------|
| REAG TEST L1: | 2.5 mL (dropper green cap) |
| REAG TEST L2-15 | 2.5 mL (dropper white cap) |
| REAG TEST LSPP | 2.5 mL (dropper black cap) |
| REAG CONTROL | 2.5 mL (dropper red cap) |
| CONTROL +: | 1.0 mL (dropper red cap) |
| SAMPLE DILUENT | 1 x 10.0 mL (vial) |
| DISPOSABLE AGGL. CARDS (SLIDE) | 35 cards with 6 wells each |
| MIXING STICKS: | 8x25 disposable mixing sticks |
| PLASTIC DROPPER | 1 item |
| INSTRUCTIONS FOR USE | 1 item |

REF 271054

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