Instructions For Use
ENGLISH

Legionella Latex Kit

For the confirmatory identification of *Legionella pneumophila*.

INTENDED PURPOSE

Latex slide agglutination test for the identification of *Legionella pneumophila* and other species of *Legionella* grown on selective media. This kit is not intended for use in the diagnosis of disease or other conditions in humans.

DESCRIPTION

This agglutination kit rapidly detects and presumptively identify *Legionella* from culture by latex agglutination. It allows the separate identification of *L. pneumophila* serogroup 1 (sg1) and serogroups 2-15* as well as other commonly isolated *Legionella* species.
*Due to cross-reactivity between serogroups 6 and 16, the kit potentially permits to detect this latter serogroup as well.

KIT CONTENTS

- Legionella Latex Reagent Sg1 (1 x 2.5 ml)
- Legionella Latex Reagent Sg2-15 (1 x 2.5 ml)
- Legionella Latex Reagent Species (1 x 2.5 ml)
- Positive Control (1 x 1.0 ml)
- 0.85% Saline (2 x 5.0 ml)
- Disposable agglutination slides (25 pcs), with 6 test areas each
- Disposable mixing sticks (75 pcs)

METHOD PRINCIPLE

Latex particles are coated with polyclonal rabbit antibodies raised against *L. pneumophila* serogroup 1 or serogroups 2-15, or other commonly isolated *Legionella* species, which are shown below. When mixed with a suspension of *Legionella* organisms or heat killed antigens from the relevant *Legionella* bacteria, the latex particles rapidly agglutinate to form visible clumps. This kit detects *L. pneumophila* and these other species of *Legionella*:

Target Legionella 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

MATERIALS REQUIRED BUT NOT PROVIDED

- Inoculating loops, pipettes, timer or clock
- Glass test tubes for boiling – Required for pretreatment if autoagglutination is seen (see TEST PROCEDURE)
- Legionella selective medium

REAGENTS

- Legionella Latex Reagent Sg1 (blue cap): Latex particles coated with rabbit polyclonal antibodies to *L. pneumophila* serogroup 1, preserved with 0.099% sodium azide.

- Legionella Latex Reagent Sg2-15 (yellow cap): Latex particles coated with rabbit polyclonal antibodies to *L. pneumophila* serogroups 2-15, preserved with 0.099% sodium azide.
- Legionella Latex Reagent Species (red cap): Latex particles coated with rabbit polyclonal antibodies to 10 commonly isolated *Legionella* species (see table above), preserved with 0.099% sodium azide.
- Positive Control (black cap): Inactivated preparation of *Legionella* antigens, preserved with 0.099% sodium azide.
- 0.85% Saline (white cap), preserved with 0.099% sodium azide.

TEST PROCEDURE

1. Allow the reagents to reach room temperature before use.
2. Dispense one drop of saline onto three circles of an agglutination slide. Using an inoculating loop, remove 3-4 colonies from the selective agar plate and emulsify the colonies in the drop of saline to produce a heavy smooth suspension. Suspensions should only be made from colonies with morphologies resembling *Legionella* spp. **Colonies should be at least 3 days old**.
3. Rock the slide gently for up to 2 minutes and observe for autoagglutination or clumping. If the suspension remains smooth, proceed to Step 4. Otherwise, proceed as outlined under LIMITATIONS, point 2.
4. Add one drop of Legionella Latex Reagent Sg1 to one of the bacterial suspensions on the agglutination slide, one drop of Legionella Latex Reagent Sg2-15 to the second suspension, and one drop of Legionella Latex Reagent Species to the third suspension. **Invert gently each latex reagent to ensure an even suspension immediately prior to use. Do not allow the dropper tip to touch the suspension on the slide.**
5. Mix the latex reagent and the bacterial suspension with a clean mixing stick for each combination and rock the slide gently two or three times. Excessive rocking of the slide is not necessary. Examine for agglutination within a maximum of 2 minutes.
6. After reading, discard the used mixing sticks and slides. Dispose of hazardous or biologically contaminated materials according to the practices of your institution.

INTERPRETING RESULTS

A positive test (+) will display agglutination, which means clumping or aggregation of the latex particles, whereas a negative result (-) will show no clumping.

Possible result patterns are shown in the following table:

Legionella Latex Reagent Sg1	Legionella Latex Reagent Sg2-15	Legionella Latex Reagent Species	Interpretation
+	-	-	Presence of *Legionella pneumophila* serogroup 1
-	+	-	Presence of *Legionella pneumophila* serogroups 2-15
-	-	+	Presence of *Legionella* spp.
-	-	-	Lack of agglutination, inconclusive result
+	+	+	Possible non-specific agglutination, inconclusive result
+	+	-	Possible non-specific agglutination, inconclusive result
+	-	+	Possible non-specific agglutination, inconclusive result
-	+	+	Possible non-specific agglutination, inconclusive result

Notes:

Inconclusive result due to the lack of agglutination does not preclude the presence of *Legionella* bacteria. The test must be repeated with additional 3-4 presumptive colonies, using increased cell mass. To achieve higher cell mass on the selective agar plate, as well as to increase expression of epitopes detected by the latex reagents, incubation time can be prolonged with an additional 24 hours.

Inconclusive result due to a non-specific agglutination pattern does not preclude the presence of *Legionella* bacteria. The test must be repeated with additional 3-4 presumptive colonies, using slightly decreased cell mass to decrease cross-reactivity caused by biomass-overload.

STORAGE

Store at 2-8°C in its original packaging. Keep away from sources of heat and avoid excessive changes of temperature. Use until the expiry date indicated on the label. Eliminate without using if there are signs of deterioration. **Do not freeze any of the kit reagents.**

USER QUALITY CONTROL

Quality control testing should be run with each shipment and new kit lot number received. It is responsibility of the individual laboratory to choose frequency and modality of QC according to internal standard operating procedures (SOPs) based on national/international guidelines.

To check the activity of the latex reagent the following controls can be performed:

1. Reagent Control: Dispense one drop of each latex reagent to separate circles on an agglutination slide. Add one drop of saline to each drop of latex reagent. Using a different mixing stick for each combination, mix and spread the liquid over the entire area of the circle. Rock the slide gently for 2 minutes and observe for agglutination. If any agglutination is seen, either the latex or the saline should be discarded.

2. Positive Control: Dispense one drop of Positive control (each kit contains sufficient reagent for 20 tests) into one circle on the agglutination slide. Add one drop of Legionella Latex Reagent Sg1 to the same circle and mix. **Do not allow the dropper tip to touch the drop of positive control on the slide**. Rock the slide gently. Within 2 minutes, agglutination, indicating a positive result, should be visible. Repeat this process using Legionella Latex Reagent Sg2-15 and Legionella Latex Reagent Species. If no agglutination is seen with any of the three latex reagents, they should be discarded, and a fresh kit should be used. **NOTE:** The reaction strength with the positive control may be not identical for the three latex reagents.

Mix the reagents by gently inverting immediately before use.

LIMITATIONS

1. Results should be interpreted in the context of all available laboratory information.
2. Old and/or mucoid cultures of *Legionella* may cause non-specific autoagglutination in saline alone (before adding the latex reagent). In that case, pre-treat as follows: Dispense 0.5 ml saline into a glass tube to 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 30 µl boiled suspension on to each of three circles of an agglutination slide. Then, go to Step 4 of TEST PROCEDURE.
3. Identification with this product is presumptive and all colonies giving positive results should be confirmed as *Legionella* spp. by their inability to grow on Legionella selective culture media deficient in cysteine.
4. 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.

WARNING AND PRECAUTIONS

For professional use only. **This kit and its reagents should not be used for clinical testing.** Operators must be trained and have certain experience. Please read the instructions carefully before using this product. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this document.

Consult the Safety Data Sheet (SDS) for information regarding hazards and safe handling practices.

Do not dilute any of the kit reagents. Do not intermix reagents from different batches of kits. Ensure the slide is clean and dry prior to use. Be careful only to record agglutination as for example reactions that are “curdy” or “stringy” may not be true agglutination.

DISPOSAL OF WASTE

Disposal of waste must be carried out according to national and local regulations in force.

BIBLIOGRAPHY

See the references at the end of this document.

TABLE OF SYMBOLS

See the table of symbols at the end of this document.

ORDER INFORMATION

Product	Packaging	Ref.
Legionella Latex Kit	50 tests	96162

Revision History

Revision	Release Date	Change Summary
0	2025-01-20	Document creation

This IFU document and the SDS are available from the online Support Center:

liofilchem.com/ifu-sds

References

1. Public Health England (2015) Identification of Legionella species. UK Standards for Microbiology Investigations. ID 18 Issue 3.
2. Katz DS (2011) Bacterial Agglutination Protocol. American Society for Microbiology (ASM).

Table of Symbols

	Batch code
	Catalogue number
	Manufacturer
	Use by
	Fragile, handle with care
	Temperature limitation
	Contains sufficient for <n> tests
	Consult instructions for use
	Do not reuse
	Keep away from sunlight

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