

LK04– HiLegionella Latex Test Kit

Product Code	Reagents provided**	LK04	
		25 Nos.	50 Nos.
LK04a	Legionella serotype 1 Latex Reagent	1.5 ml	3.0 ml
LK04b	Legionella serotype 2-15 Latex Reagent	1.5 ml	3.0 ml
LK04c	Positive Control	0.5 ml	1.0 ml
LK04d	Sample diluent	2.5 ml	5.0 ml

** Agglutination slides and mixing sticks are provided in the kit.

Intended Use

HiLegionella Latex Test Kit is a latex agglutination test intended for confirmatory identification of *Legionella pneumophila* grown on selective media. The test is suitable for organisms derived from patients with suspected Legionella pneumonia or from environmental sources. HiLegionella Latex Test Kit allows the separate identification of *L.pneumophila* serogroup 1 and serogroups 2-15*.

*Note: *L.pneumophila* serogroup 15 is still awaiting official ratification.

The kit is intended for *in vitro* diagnostic use only. Not for Medicinal Use.

Principle of the Test

For Legionella 1 Latex Reagent, latex particles are coated with polyclonal rabbit antibodies raised against *L.pneumophila* serogroup 1 enhanced with mouse monoclonal antibody coated latex particles. For Legionella serotype 2-15 Latex Reagent, latex particles are coated with polyclonal rabbit antibodies raised against serogroups 2-15. When these latex particles are mixed with a suspension containing *L.pneumophila* antigens, 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 antigen. Similarly, 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.

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 monoclonal antibody enhancement of Test Reagent 1 and the dilution effect of blending 15 different antisera during manufacture of Test Reagent 2-15.

Kit Contents

1. LK04a Legionella serotype 1 Latex Reagent :

1

Latex particles coated with rabbit polyclonal and mouse monoclonal antibodies to L.pneumophila serogroup 1. Preserved with 0.099% sodium azide.

2. LK04b Legionella serotype 2-15 Latex Reagent :

Latex particles coated with polyclonal rabbit antibodies to L.pneumophila serogroups 2-15. Preserved with 0.099% sodium azide.

3. Positive Control

Suspension of inactivated Legionella antigens reactive with both Test Reagents 1 and 2-15. Preserved with 0.099% sodium azide.

4. Sample diluent

Preserved with 0.099% sodium azide.

Instructions for Use

- Disposable agglutination slides
- Disposable mixing sticks

Additional Requirement

- Bacteriological loops (PW012 Hi-FlexiLoop 2).
- Micropipettes and tips
- Legionella selective medium (Legionella Agar Base-M809 with FD016A*, FD017*, FD041A*)
Note- FD016A*, FD017*, FD041A* - For more details refer HiMedia Product Manual.
- Glass tubes for boiling
- 0.85% isotonic saline

Warnings

Safety:

1. The reagents supplied in this kit are for *in vitro* diagnostic use only.
2. 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.
3. The positive control has been inactivated during the manufacturing process. However, it should be handled as though potentially infectious.

Specimen Collection and Handling

Colonies grown on selective agar plates (Legionella Agar Base-M809 with FD016A*, FD017*, FD041A*) can be tested with HiLegionella Latex Test Kit. The morphology of the colonies tested should resemble that of Legionella.

Note- FD016A*, FD017*, FD041A* - For more details refer HiMedia Product Manual.

Procedure

1. HiLegionella Latex Test Kit should be used according to the kit instructions.
2. Allow all reagents to reach room temperature before use.
3. Do not dilute any of the kit reagents.
4. Do not intermix reagents from different batches of kits.
5. Do not freeze any of the kit reagents.
6. Be careful only to record agglutination. Reactions that are “curdy” or “stringy” may not be true agglutination.
7. Ensure the agglutination slide is clean and dry prior to use.

Storage and Shelf Life

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

Quality Control

Organism	Agglutination with latex Reagent
<i>Legionella pneumophila</i>	+
Key : + is agglutination , - is no agglutination	

Performance & Evaluation

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

1. Reagent Control:

Gently mix the HiMedia test latex reagents (LK04a, LK04b) and add 20µl of each reagent to separate wells on the agglutination slide. Add 20µl of saline solution (LK04d) to each 20µl 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 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.

2. Positive Control:

Add 20µl of positive control (LK04c) to one well on the agglutination slide. Gently mix Legionella 1 Latex Reagent (LK04a) and add 20µl to the same well. 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 Legionella serotype 2-15 Latex Reagent (Lk04b). Again, a positive result should be seen. If agglutination is not seen with either of the latex reagents, a fresh kit should be used.

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

Test Procedure:

1. Dispense 20µl of isotonic saline (LK04d) on to each of two wells of the agglutination slide.
2. Using an inoculating loop, remove several Legionella-like colonies from the selective agar plate and make a thick even smear on the slide alongside each 20µl drop of saline.
3. Mix the colonies with the saline and emulsify to form a smooth heavy suspension, spreading the liquid over the entire surface of the well.
4. Rock the slide gently for up to 2 minutes and observe for autoagglutination or clumping. If the suspension remains smooth, proceed to point 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 of 0.85% isotonic saline into a glass tube. Prepare a homogeneous turbid suspension of organisms taken from the selective agar plate. (Legionella Agar Base- M809 plus FD016A*, FD017*, FD041A*) Note- FD016A*, FD017*, FD041A* - For more details refer HiMedia Product Manual.
6. Boil the suspension for 5 minutes. Allow to cool to room temperature. Place 30 µl boiled suspension on to each of two wells of an agglutination slide.

7. Gently mix each test latex reagent (LK04a, LK04b) to ensure a homogeneous suspension.
8. Add 20 µl of Legionella 1 Latex Reagent to one of the bacterial suspensions and 20 µl of Legionella serotype 2-15 Latex Reagent to the other 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. Rock the slide gently for 2 minutes and observe for agglutination.
11. Discard the used mixing sticks and slides into a suitable disinfectant.

Interpretation

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

Reaction with Legionella 1 Latex Reagent	Reaction with Legionella serotype 2-15 Latex Reagent	Interpretation
+	-	L. pneumophila 1 present
-	+	L. pneumophila 2-15 present
-	-	L. pneumophila 1- 15 not present
+	+	Possible non-specific agglutination. Inconclusive result*

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

Limitations of Use

1. Results should be interpreted by the clinician in the context of all available clinical and laboratory information.
2. Stringy reactions on the slide 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 pretreated by boiling as described in the method above.
4. HiLegionella Latex Test Kit is intended for the identification of L.pneumophila serotypes 1-15 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.

Precautions

Read the procedure carefully before starting the experiment.

Performance Characteristics

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

	HiLegionella Latex Test Kit (All serogroups)			Total
		+ve	-ve	
Commercial Latex Test (All serogroups)	+ve	50*	0	50
	-ve	0	78	78
	Total	50	78	128

Sensitivity: 50/50 = 100%

Specificity: 78/78 = 100%

Accuracy: 128/128 = 100%

70 isolates of *C* and Legionella spp. were tested in both HiLegionella and the comparison latex kit to differentiate between Serogroup 1 and Serogroups 2-15.

*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), *Strep* spp. (1). However, all of the above either do not grow or show very atypical morphologies, when cultured on Legionella selective media. In the case of *B. cereus*, agglutination was atypical (stringy).

		HiLegionella Latex Test Kit			Total
		SG1 Positive	SG 2-15 Positive	<i>L. pneumophila</i> negative	
Commercial Latex Test	SG1 Positive	8	0	0	8
	SG 2-15 Positive	0	30	0	30

	L. pneumophila negative	0	0	32	32
	Total	8	30	32	70

No isolates of L. pneumophila serogroup 1 cross-reacted with Test Reagent 2-15. Similarly, no isolates of L. pneumophila serogroups 2-15 cross-reacted with Test Reagent 1. All nonpneumophila Legionella spp isolates were non-reactive with both test latex reagents in HiLegionella Latex Test Kit.

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%.

Disposal

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 neutralized before treatment.

Technical Assistance

At HiMedia, we pride ourselves on the quality and availability of our technical support. For any kind of technical assistance, mail to mb@himedialabs.com.



In vitro diagnostic medical device



CE Marking



Consult instructions for use

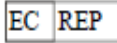


Do not use if package is damaged



HiMedia Laboratories Pvt. Limited,
Reg. Off: 23 Vadhani Industrial Estate,
LBS Marg, Mumbai - 400086, India
Works: B/4-6, M.I.D.C., Dindori, Nashik, India
Customer Care No: 022-6116 9797
Email : techhelp@himedialabs.com

www.himedialabs.com



CE Partner 4U ,Esdoomlaan
13, 3951 DB Maarn The
Netherlands,
www.cepartner4u.eu

PILK04_O/1216

LK04-04

Disclaimer :

User must ensure suitability of the product(s) in their application prior to use. Products conform solely to the information contained in this and other related HiMedia™ publications. The information contained in this publication is based on our research and development work and is to the best of our knowledge true and accurate. HiMedia™ Laboratories Pvt Ltd reserves the right to make changes to specifications and information related to the products at any time. Products are not intended for human or animal diagnostic or therapeutic use but for laboratory, research or further manufacturing use only, unless otherwise specified. Statements contained herein should not be considered as a warranty of any kind, expressed or implied, and no liability is accepted for infringement of any patents.

HiMedia Laboratories Pvt. Ltd. A-516, Swastik Disha Business Park, Via Vadhani Ind. Est., LBS Marg, Mumbai-400086, India. Customer care No.: 022-6147 1919 Email: techhelp@himedialabs.com Website: www.himedialabs.com