Instruction for Use

Microgen *Legionella* Latex Kit

Cat. No. M45

GOLD STANDARD DIAGNOSTICS

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1 INTRODUCTION

Microgen 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 samples with suspected *Legionella pneumonia* or from environmental sources. Microgen Legionella allows the separate identification of *L. pneumophila* Serogroup 1 and Serogroups 2-15* and commonly isolated *Legionella* species.

***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.

The kit is intended for professional use only, to be used for industrial diagnostics only and not for use in clinical testing.

2 PRINCIPLE

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

3 REAGENTS

Kit Contents (50 tests)

- Test Reagent 1: **M45a** / 2.5ml / blue cap
  Latex particles coated with rabbit polyclonal antibodies to *L. pneumophila* serogroup 1. Preserved with 0.099% sodium azide.
- Test Reagent 2-15: **M45b** / 2.5ml / yellow cap
  Latex particles coated with polyclonal rabbit antibodies to *L. pneumophila* serogroups 2-15. Preserved with 0.099% sodium azide.
- Positive Control: **M45c** / 1.0ml / black cap
  Suspension of inactivated *Legionella* antigens reactive with Test Reagents 1, 2-15 and Species. Preserved with 0.099% sodium azide.
- Test Reagent Species: **M45d** / 2.5ml / red cap
  Latex particles coated with polyclonal rabbit antibodies to 10 commonly isolated *Legionella* species (see table above).
  Preserved with 0.099% sodium azide.
- 0.85% Isotonic Saline: **M40** / 2 x 5.0ml / white cap
  Preserved with 0.099% sodium azide.
- Disposable agglutination slides (25pcs)
- Disposable mixing sticks (3x25pcs)

Additional Materials Required (not supplied in the kit)

- Bacteriological loops
- Legionella selective medium
- Glass tubes for boiling
- 0.85% isotonic saline

4 STORAGE

Microgen Legionella should be stored at 2-8ºC when not in use. The kit should not be used after the expiry date printed on the carton label.

5 TEST STEPS

Before using this product, refer to Precautions and Limitations. Please note, the controls specified in Section 8 should be performed each time the kit is used.

SPECIMENS

Colonies grown on selective agar plates can be tested with Microgen Legionella. The morphology of the colonies tested should resemble that of *Legionella*. **Colonies should be at least 3 days old.**

TEST PROCEDURE:

1. Dispense 1 drop of isotonic saline (M40) on to each of three wells of the agglutination slide.
2. Using an inoculating loop, remove 3-4 *Legionella*-like colonies from the selective agar plate and make a thick even smear on the slide alongside each 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. If the suspension remains smooth, proceed to section 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 each test 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. 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. Microgen Legionella results should be interpreted as follows:

Test Reagent 1	Test Reagent 2-15	Test Reagent Species	Interpretation
+	-	-	*L. pneumophila* 1 present
-	+	-	*L. pneumophila* 2-15 present
-	-	+	*Legionella* species present
-	-	-	Lack of agglutination, inconclusive result*
+	+	+	Possible nonspecific agglutination, inconclusive result**
+	+	-	Possible nonspecific agglutination, inconclusive result**
+	-	+	Possible nonspecific agglutination, inconclusive result**
-	+	+	Possible non-specific agglutination. Inconclusive result**

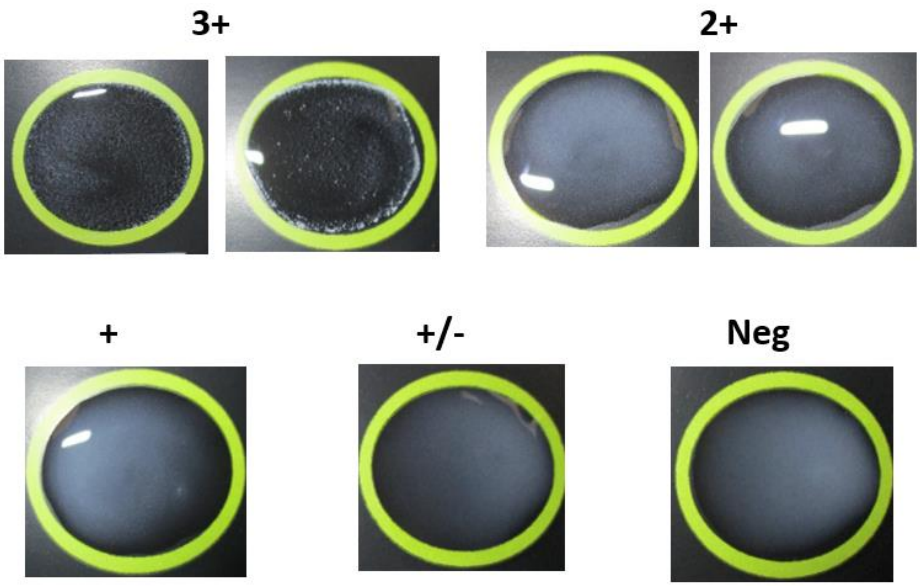
* The lack of agglutination for presumptive *Legionella* colonies grown on a selective agar plate does not preclude the presence of *Legionella* bacteria but results have to be interpreted as inconclusive. The test must be repeated with additional 3-4 presumptive colonies, using increased cell mass. 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.

**A non-specific agglutination pattern does not preclude the presence of *Legionella* bacteria, but results have to be interpreted as inconclusive. The test must be repeated with additional 3-4 presumptive colonies, using slightly decreased cell mass to decrease cross-reactivity caused by biomass-overload.

REACTION GRADE PATTERN

Reaction grade	Description
3+	Large, agglutinated particles, which may form a ring of white precipitation. Background is clear.
2+	Visible agglutination, but background appears milky.
+	Fine agglutination where the particles are seen only when rocking. Background appears milky.
Trace (Tr +/-)	Very fine agglutination only seen when rocking with a milky background. A middle ground between + reaction and a negative reaction.
Negative (-)	No agglutination, appears as a milky liquid.

Figure 1 – Reaction grade pattern examples



6 PRECAUTIONS

Safety:

1. The kit is intended for professional use only, to be used for industrial diagnostics only and not for use in clinical testing.

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. 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.
4. The positive control has been inactivated during the manufacturing process. However, it should be handled as though potentially infectious.

Procedural:

1. Microgen Legionella 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 inter mix reagents from different batches of kits.
5. Do not freeze any of the kit reagents.
6. Do not allow the latex reagent dropper to touch the positive control or bacterial samples.
7. Be careful only to record agglutination. Reactions that are “curdy” or “stringy” may not be true agglutination.
8. Ensure the agglutination slide is clean and dry prior to use.

7 LIMITATIONS

1. Results should be interpreted in the context of all available 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 pre-treated by boiling as described in the method above.
4. Microgen Legionella 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.
5. 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.

8 QUALITY CONTROL

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

1. **Reagent Control**: Gently mix the Microgen test latex reagents (M45a, M45b, M45d) and add one drop of each reagent to separate wells on the agglutination slide. Add one drop of saline solution (M40) 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 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 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 above)

9 WASTE DISPOSAL

Dispose of according to any local, national, or regional regulations.

10 PRODUCT WARRANTIES, SATISFACTION GUARANTEE

Gold Standard Diagnostics Budapest ("GSDB") warrants that the products manufactured by it will be free of defects in materials and workmanship, when used in accordance with the applicable instructions before the expiration date marked on the product packaging, and when stored under the storage conditions recommended in the instructions and/or on the package.

GSDB makes no other warranty, expressed or implied.

GSDB's sole obligation shall be, at its option, to either replace or to refund the purchase price of the product(s) or part there of that proves defective in materials or workmanship within the warranty period, provided the customer notifies GSDB promptly of any such defect within a reasonable time and with solid proof of the defect. GSDB shall investigate the defect locally and will justify the approval or disapproval of the complaint.

GSDB shall not be liable for any direct, indirect or consequential damages resulting from economic loss or property damages sustained by buyer or any customer from the use of the product(s).

A copy of the terms and conditions can be obtained on request and is also provided in our price lists.

TECHNICAL SUPPORT SERVICE

For technical assistance and more information please contact Gold Standard Diagnostics Budapest's Customer Service or your local distributor.

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LIST OF MODIFICATIONS		
VERSION	DESCRIPTION OF THE CHANGE	ISSUE DATE
1.0	First issue	12 SEP 2023
2.0	Second issue: Change of result interpretation of the lack of agglutination with all 3 latex reagents (Serogroup 1, Serogroup 2-15 and L. spp) from „No Legionella present“ to „Lack of agglutination, inconclusive result“. Addition of the need of a re-test in case of an inconclusive result. Additional instructions on how to carry out the re-tests to decrease the possibility of an inconclusive result	15 JUL 2024
3.0	Third issue: Removal of the Microgen logo	25 APR 2025