# PathFlow™ Legionella pneumophila Rapid Test Cassette (EN)

MICROGEN BIOPRODUCTS

## Instructions for Use

### INTENDED USE

The PathFlow™ *Legionella pneumophila* Rapid Test Cassette (Urine) is a rapid chromatographic immunoassay for the qualitative detection of *Legionella pneumophila* serogroup 1 in human urine specimen.

### SUMMARY

Legionellosis is a serious pneumonia caused by bacteria of the genus Legionella, assigned to the family Legionellaceae. This family now includes 48 species and over 60 serogroups, approximately 20 species are implicated in human disease. Most Legionella infections are caused by *Legionella pneumophila*. Legionnaires' disease is the major clinical manifestation of Legionella infection although extra-pulmonary infection and non-pneumonic disease like Pontiac fever can occur. The name Legionella pneumophila was derived from the dramatic outbreak at the 1976 American Legion Convention in Philadelphia. [1]

*Legionella pneumophila* is responsible for approximately 90% of infections, and of these, over 80% are due to a single serogroup, serogroup 1. [2] Legionella bacteria are small, faintly staining Gram-negative rods with polar flagella. Legionella bacteria have a widespread distribution in both natural and manmade aquatic habitats, they are readily found in fresh water, cooling towers and portable water systems. The organisms can survive in a wide range of conditions; however, temperature is a critical determinant for Legionella proliferation. Nosocomial infection is particularly associated with colonization of hospital hot water system by Legionella.

The incubation period of Legionnaires' disease after being exposed to the bacteria is from two to ten days. Most patients who are admitted to the hospital develop high fever often higher than 39.5°C (103°F). A cough can be the first sign of a lung infection, other common symptoms include: headaches, muscle aches, chest pain, and shortness of breath. Gastrointestinal symptoms are also common.

Legionnaires' disease (LD) is not contagious. The disease is transmitted by aerosol, and there is no evidence for direct person-to-person transmission. People at risk are those whose immune system is compromised, including transplant recipients, the elderly, smokers, or those showing chronic obstructive pulmonary disease or chronic renal disease. [3]

Diagnosis of legionellosis can be difficult because signs and symptoms are nonspecific and do not distinguish *L. pneumophila* infections from other common causes of pneumonia. *L. pneumophila* infections are considered fairly common, but they are probably underdiagnosed and under-reported. The underdiagnosis of legionellosis can in part be attributed to the need for rapid, specific and sensitive diagnostic testing methods.

PathFlow™ *Legionella pneumophila* Rapid Test Cassette (Urine) detects soluble antigen from *L. pneumophila* serogroup 1 in urine. [2]

### PRINCIPLE

The PathFlow™ *Legionella pneumophila* Rapid Test Cassette (Urine) is a ready-to-use membrane test based on colloidal gold particles, allowing for the detection of *Legionella pneumophila* in urine samples.

The test sensitivity and specificity are determined by monoclonal and polyclonal anti-Legionella antibodies. Mouse anti-Legionella antibodies are conjugated to colloidal gold particles and dried on a conjugate absorbent pad. Each strip is sensitized with goat anti-Legionella antibodies at the T-line region and with a control antibody at the C-line region. When the urine sample migrates, conjugate is rehydrated and migrates along with the sample. If *L. pneumophila* urinary antigens are present in the sample, a complex between the anti-*L. pneumophila* conjugates and the *L. pneumophila* antigens is formed that will be caught by the specific anti- *L. pneumophila* reagent coated on the membrane. Results appear in 15 minutes in the form of a red line that develops on the strip.

### REAGENTS

The test cassette contains mouse anti-Legionella particles and goat anti-Legionella coated on the membrane.

### MATERIALS

Provided: Test cassettes, Package insert, Droppers.

Not Provided but required: Timer.

### WARNINGS AND PRECAUTIONS

- For professional in vitro diagnostic use only. Do not use after expiration date.
- The test cassette should remain in the sealed pouch until use.
- Do not eat, drink or smoke in the area where the specimens or kits are handled.
- Handle all specimens as if they contain infectious agents. Observe established precautions against microbiological hazards throughout all procedures and follow the standard procedures for proper disposal of specimens.
- Wear protective clothing such as laboratory coats, disposable gloves and eye protection when specimens are assayed.
- The used test materials should be discarded according to local regulations.
- Humidity and temperature can adversely affect results.

### STORAGE AND STABILITY

- Store as packaged at room temperature or refrigerated (2-30°C).
- The test is stable until the expiration date printed on cassette pouch.
- The test must remain in the sealed pouch until use.
- **DO NOT FREEZE**
- Do not use beyond the expiration date.

### SPECIMENS

It is advised the test is to be used in conjunction with freshly collected samples to provide optimal results. Inadequate sample collection or improper sample handing could potentially yield a false-negative result.

Ensure that the specimen(s) are not treated with cleaning solutions containing formaldehyde or its derivatives.

Specimens to be tested should be obtained and handled by standard methods for the collection of urine sample. Urine specimens should be collected in standard containers - the use of Proclin 300 as preservative has been validated on the PathFlow™ *Legionella pneumophila* Rapid Test Cassette.

Urine sample specimens must be tested as soon as possible after they are collected. If necessary, they can be stored at 2-8°C for up to 1 week or at -10°C to -20°C for longer periods of time.

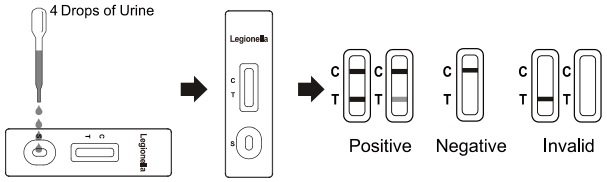
Although it requires added processing time, the antigens present in the urine can be concentrated with a disposable concentrator or a centrifugation system.

### DIRECTIONS FOR USE

**Allow the test, specimen, buffer and/or controls to reach room temperature (15-30°C) prior to testing.**

Remove the test cassette from the sealed foil pouch and use it as soon as possible. Best results will be obtained if the assay is performed immediately after opening the foil pouch.

1. Swirl the urine sample gently to mix before testing.
2. Add 4 drops of homologised urine sample (approximately 100 µL) to the sample well.
3. Wait for the colour line to appear. Read the results at 15 minutes, do not interpret the results after 20 minutes.




### INTERPRETATION OF RESULTS

**POSITIVE: *Two distinct coloured lines appear.** One coloured line should be present in the control line region (C) and another in the test region (T).

***NOTE:** The intensity of the colour in the test line region (T) will vary depending on the concentration of test antigens present in the specimen.

*Any shade of colour in the test line region should be considered positive.*

**NEGATIVE: One coloured line appears in the control line region (C).** No line appears in the test line region (T).

**INVALID: Colour line (C) fails to appear.** Insufficient specimen volume or incorrect procedural technique are the most likely reasons for control line failure.

*Review the procedure and repeat the test with a new cassette.*

*If the problem persists, discontinue using the cassette immediately and contact your local distributor.*

### QUALITY CONTROL

Internal procedural controls are included in the test. A coloured line appearing in the control region (C) is an internal valid procedural control. It confirms that sufficient specimen volume has been used and correct procedural technique has been followed.

Control standards are not supplied with this kit; however, it is recommended that positive and negative controls be tested as a good laboratory practice to confirm the test procedure and to verify proper test performance.

### LIMITATIONS

1. The PathFlow™ *Legionella pneumophila* Rapid Test Cassette (Urine) is for in vitro diagnostic use only. Neither the quantitative value nor the rate of increase in Legionella antigen concentration can be determined by this qualitative test.
2. The PathFlow™ *Legionella pneumophila* Rapid Test Cassette (Urine) will only indicate the presence of *Legionella pneumophila* in the specimen and should not be used as the sole criteria for diagnosis.
3. As with all diagnostic tests, all results must be interpreted together with other clinical information available to the physician.
4. If the test result is negative and clinical symptoms persist, additional testing using other clinical methods is recommended. A negative result does not at any time preclude the possibility of legionella infection.

5. The PathFlow™ *Legionella pneumophila* Rapid Test Cassette (Urine) is an acute-phase screening test for qualitative detection. Sample collected may contain antigen titles below the reagent's sensitivity threshold, so a negative test result does not exclude infection with Legionella.
6. The PathFlow™ *Legionella pneumophila* Rapid Test Cassette (Urine) detects both viable and non-viable legionella antigens. Test performance depends on antigen load in the sample and may not correlate with cell culture performed on the same sample. A positive test does not rule out the possibility that other pathogens may be present. Therefore, the results must be compared with all other available clinical and laboratory information to make an accurate diagnosis.

**PERFORMANCE CHARACTERISTICS**

The PathFlow™ *Legionella pneumophila* Rapid Test Cassette (Urine) was evaluated on 109 clinical samples in a National Reference Laboratory in Spain. 41 urine samples from patients with LD defined by clinical and radiological signs of pneumonia and microbiologically confirmed were studied. EIA method was used as laboratory evidence. Urine samples from patients with respiratory tract infections other than Legionella infections were tested in a similar manner to test the specificity of the kit.

| **Method** | | **EIA** | | **Total Result** |
|---|---|---|---|---|
| **PathFlow™ Legionella** | **Results** | Positive | Negative | |
| | Positive | 40 | 0 | 40 |
| | Negative | 1 | 68 | 69 |
| **Total Result** | | 41 | 68 | 109 |

Relative sensitivity: 97.6% (95%CI*: 87.1%~99.9%);

Relative specificity: >99.9% (95%CI*: 95.7%~100%);

Accuracy: 99.1% (95%CI*: 95.0%~99.9%).

*Confidence Intervals

The results show that the relative sensitivity of PathFlow™ *Legionella pneumophila* Rapid Test Cassette (Urine) is 97.6%, relative specificity is >99.9% and the relative accuracy is 99.1%.

**Repeatability and reproducibility**

To check intra-batch accuracy (repeatability), same positive and negative urine samples have been processed 15 times on kits of the same production batch in the same experimental conditions. The samples produced the expected results in 100% of cases.

To check inter-batch accuracy (reproducibility), same samples (positive and negative) were processed on kits from three different production batches. The samples produced the expected results in 100% of cases.

**CROSS REACTIVITY**

Cross-reactivity to urine spiked with the following pathogens was tested and found to be negative:

Adenovirus, Clostridium difficile, HMPV, Aspergillus niger, E.coli (different strains), Streptococcus mutans, Candida albicans, Enterobacter cloacae ,Vibrio parahaemolyticus, Haemophilus influenzae, Enterococcus faecalis, Ureaplasma urealyticum, Influenza A+B, Escherichia hermanni, Mycobacterium avium, Helicobacter pylori, Mycobacterium intracellulare, Moraxella catarrhalis, Klebsiella pneumoniae, Mycobacterium tuberculosis, Mycoplasma pneumonia, Legionella bozemanii (sg1), Serratia marcescens, Nocardia asteroide, Legionella longbeachae, Pseudomonas aeruginosa, Parainfluenzae, Neisseria meningitidis, Shigella sonnei, Rhinovirus, Proteus mirabilis, Campylobacter coli, RSV, Salmonella enteritidis, S. typhimurium, Staphylococcus aureus, Shigella flexneri, Vibrio parahaemolyticus, Streptococcus pneumonia, Staphylococcus epidermidis, Neisseria meningitidis (sg C), Streptococcus pyogenes,Yersinia enterocolitica (types 3,9), Mycoplasma hominis, Campylobacter jejuni, Streptococcus (Group B, C, F, G).

The blood naturally present in urine (microhaematuria conditions) doesn't affect test performances. However, bloody specimens (at 0.1% whole blood) may fail to flow properly causing smears and inconclusive test results.

**INTERFERING SUBSTANCES**

The following analytes were tested as interfering substances spiked with negative and middle positive samples: Acetaminophen (20mg/dl), Acetoacetic Acid (2000mg/dl), Ascorbic Acid (20mg/dl), Acetylsalicylic Acid (20mg/dl), Albumin (2000mg/dl), Bilirubin (2mg/dl), Caffeine (20mg/dl), Codeine (10mg/dl), Ephedrine (20mg/dl), EDTA (20mg/dl), Ethanol (1%), Gentisic Acid (20mg/dl), Glucose (2000mg/dl) haemoglobin (1mg/dl), Methadone (10mg/dl), Methanol (10%), Phenylpropanolamine (20mg/dl), Phenothiazine (20mg/dl), Salicylic Acid (20mg/dl). No substances showed any interference with the test.

**LIMIT OF DETECTION**

The detection level of the PathFlow™ Legionella pneumophila rapid test can detect Legionella pneumophila antigen as low as 50ng/ml.

**BIBLIOGRAPHY**


1. B. M.W. Diederen; Legionella spp. and Legionnaires' disease; J. Inf. 2008 56:1-12, 2008.
2. J.H. Helbig et al.; Pan-European study on culture-proven Legionnaires' Disease; Eur. J. Clin. Microbiol. Infect. Dis. 2002 21:710-716, 2002.
3. B.S. Fields et al.; Legionella and Legionnaires' disease: 25 years of investigation; Clin. Microbiol. Rev. 2002 15: 506-526, 2002.


**TABLE OF SYMBOLS**

| SYMBOL | DEFINITION |
|---|---|
|  | Batch Code/Lot Number |
|  | In Vitro Diagnostic Medical Device |
|  | Catalogue Reference |
|  | Upper and Lower Temperature Limits |
|  | Expiry Date |
|  | Manufacturer and Address Details |
|  | Date of Manufacture |
|  | Please consult IFU |
|  | Sufficient for |
|  | Do not use if package is damaged |
|  | CE Mark |
|  | Caution |

**This document applies to the following product codes:**
**M593CE**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