Lovibond® Water Testing

Tintometer® Group



Enterprise Test Kit



Legionella pneumophila serogroup 1 in Water & Biofilms

Instruction Manual Part Number: 56B006501

www.lovibond.com

Technical Information

Overview

This test kit is used to detect the presence of *Legionella pneumophila* serogroup 1 bacteria in hot and cold water systems, cooling water, whirlpool spas and in biofilm. The test operates via a Lateral Flow Immuno-Chromato-graphic Assay (LFICA).

Each Enterprise Test Kit contains the following:

- 4 Individually foil -wrapped tests -- each contains an exact volume pipette
- 2 Hollow Fiber Filters
- 2 Syringes with Recovery Buffer
- 2 Swabs
- 2 Large Sample Syringes (60 ml)
- 2 Re-suspension tubes with Recovery Buffer Solution
- Instructions for use

This test is intended for analysis of water samples only. This product is not intended for clinical or medical diagnostic use.

The product is intended to be used as part of an overall water treatment, management, and risk reduction approach and should NOT be used as the sole method for assessing risks associated with Legionella bacteria.

Test Operating Limits

The test has been evaluated for operation between 10-40°C (50-104°F).

The test has been validated for samples that filter in less than 10 minutes. Samples requiring more than 10 minutes to filter may give erroneous results. Samples that require long times to sample may be indicative of poor system maintenance. A wide range of non-oxidizing biocides and biodispersants have been checked for cross reaction and inference with the test. **This test should not be used on systems treated with biguanide or THPS based biocides!**

Performance of the test with samples other than those taken from Cooling Towers, Domestic Hot & Cold water systems and Whirlpool spas has not been established.

Limit of Detection - Filtered Test for Water Samples

Laboratory analysis has demonstrated that 98-100% (91% CI) of tests are positive for clean water samples containing 100 CFU/Liter *Legionella pneumophila* serogroup 1. The theoretical mathematical limit of detection of the test is equivalent 100 CFU/L when a 250 ml sample is filtered. If smaller volumes are processed, the detection limit will be altered accordingly: 100 CFU

limit of detection =

Sample Volume (L)

Suspended solid content in the water samples impacts filtration and test performance, including analytical sensitivity. Acutual results will vary. Water samples with high levels of suspened solids may block filtration entirely. *Legionella pneumophila* serogroup 1 bacteria recovery from water samples can range from <10 to 100%, depending on water sample composition. This is similar to filtration concentration techniquest used in other microbiological analysis methods.

Limit of Detection - Swab Test for Biofilms

The mathematical theoretical limit of detection of the test is equivalent to 200 CFU / swabbed area.

Specificity

The test has been shown to be non-reactive with the following bacteria (at 1 x 10⁸ organisms per sample):

Acinetobacter calcoaceticus, Aeromonas hydrophila subsp. hydrophila, Bacillus subtilis, Burkholderia cepacia, Citrobacter freudii, Citrobacter Koseri,

Escherichia coli, Enterobacter cloacae, Klebsiella oxytoca, Pseudomonas aeruginosa, Pseudomonas fluorescens, Pseudomonas putida, Pseudomonas stutzeri, Ralstonia pickettii, Raoultella terrigena, Streptococcus pyrogenes, Yersinia ruckeri

Staphylococcus aureus and Legionella pneumophila serogroups 4 & 7 in concentrations higher than 1x10⁸ organisms per sample may interfere with test results in negative samples. These concentrations are higher than would be expected to be present in normal water samples.

Disposal

The test, filter, syringe and caps cannot be reused or recycled. The packaging materials and this instruction manual can be recycled.

Storage

The test is intended for storage at room temperature. Do Not Freeze. When stored correctly, the test will continue to operate within design specifications, until the specified expiration date. **Do not use the test after the stated expiration date specified on the packaging**, **Do not use any test where the foil packaging is damaged.**

Test Procedure - Swab Test for Biofilms



Collect Biofilm Sample

Identify the water system to be sampled and an appropriate location from which to obtain a biofilm sample. Large systems may need to be sampled and tested at multiple locations. The recommended minimum area to swab is 10 cm². If the surface to be sampled is dry, then pre-moisten the swab in recovery buffer, using the resuspension tube. Wipe the swab across the area to be tested.

Avoid generating aerosols when collecting or handling samples!

Transfer the swab containing sample to the re-suspension tube and snap off the handle.

Resuspend the Bacteria

Add 2 ml of Recovery Buffer to the re-suspension tube containing the swab sample and screw on the lid. Shake the tube for at least 20 seconds or until the swab has released the biofilm sample into the recovery buffer.

Add Sample to the Test Strip

Remove the test from its foil wrapping, and place the test on a flat surface.

The foil wrapping should not be opened until immediately prior to running the test. If the foil is opened and then the test is not performed within 60 minutes, discard the test.

Before use, the test should have 2 pale blue lines across the result window. If these are not present, notify your supplier to replace the test kit. Take the pipette from the foil wrapping.

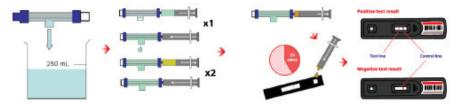
Place open end of the pipette into the solution in the re-suspension tube, then squeeze and release the top bulb. This should draw the sample all the way up the long tube and may place a small amount of sample in the bottom of the bulb. Avoid getting air bubbles in the tube -- the pipette filling process can be repeated if necessary to remove air bubbles.

Place the pipette over the small sample window at the end of the test strip, and squeeze the pipette bulb to dispense the correct amount of water onto the test strip.

Record the time.

For how to read and interpret the test results, see page 6.

Test Procedure - Filtered Test for Water Samples



Take the Sample

Before taking the sample, flush the sample port for 15 seconds. Collect a water sample of at least 250 ml in a clean container.

From the kit, take a 60 ml syringe and draw up 50-60 ml of the sample. Remove the Hollow Fiber Filter from the packaging and attach it to the luerlock edge of the syringe. Filter the sample over a sink or other waste water outlet. Repeat this process until all of the original 250 ml sample has been filtered. This should take no longer than 10 minutes.

Resuspend the Bacteria

Disconnect the filter from the syringe. Disgard the large syringe. The hollow fiber filter has a white cap on the opposite end of the side used for attaching to the syringe. Remove this cap and swap ends, so that the cap is now screwed into the place where you attached the sampling syringe. by twisting the lock ring and pulling the filter from the fitting.

Now, take a small syringe of recovery buffer, remove the red cap and attach it to the open end of the hollow fiber filter.

- Pull the syringe back to the 0.5 ml mark to suspend the recovery buffer, then push the syringe all the way to the 0 ml mark.
- Repeat this process two (2) additional times.
- Draw the syringe back to collect 0.1 ml of sample, then disconnect from the filter. The syringe
 now conains the recovered bacteria ready for testing.

Add Sample to the Test Strip

Remove the test from its foil wrapping, and place the test on a flat surface.

The foil wrapping should not be opened until immediately prior to running the test. If the foil is opened and then the test is not performed within 60 minutes, discard the test.

Before use, the test should have 2 pale blue lines across the result window. If these are not present, notify your supplier to replace the test kit. Take the pipette from the foil wrapping.

Place the syringe over the small sample window at the end of the test strip, and press the plunger so that 0.1 ml of sample is dispensed onto the test strip. **Record the time.**

For how to read and interpret the test results, see page 6.

Test Procedure, continued.

Read the Test Strip

Leave the test strip sitting on a flat surface during incubation. For optimum results the test results should be performed at room temperature. **After waiting 25 minutes, examine the test strip** in good lighting. If the test is not read within 60 minutes of adding the water sample, it should be discarded and a new test should be run. The test should show one of the following results on the large result window on the test strip:

Negative Result: One (1) RED line across the result window at the end furthest from the sample window.

Positive Result: Two (2) RED lines across the result window. The red line closest to the sample window may be very faint (pale pink). Any distinct line, no matter how faint, should be counted as positive.

Invalid Result: If the test does not show any red lines; or if it only shows a line at the end closest to the sample window; or if the line furthest from the sample window is very faint, then the test is invalid. Repeat the test and notify your supplier for technical support.

Example of a new test





Example of a positive test

Example of a negative test



Interpreting the Results

Positive Results:

A positive test result indicates that *Legionella pneumophila* serogroup 1 bacteria were present in the sample above the detection limit.

The test does not differentiate between viable (living) and non-viable (dead) organisms. The test will detect viable but non-cultruable bacteria which are not detectable by traditional laboratory techniques. A positive result does not necessarily mean that viable bacteria are present.

When a positive result is observed, seek advice from your risk management plan, or water treatment specialist.

Negative Results:

A negative test result indicates that *Legionella pneumophila* serogroup 1 bacteria were not detected or the number of bacteria in the sample were below the detection limit.

A negative result does not necessarily mean that Legionella bacteria are absent.

A negative result does not mean that the system is completely free from risks associated with *Legionella* bacteria. The test only detects *Legionella pneumophila* serogroup 1. The test does not detect the presence of other *Legionella* species or serogroups.

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