Limit of detection

Laboratory analysis has demonstrated that tests are positive for clean water samples containing 1000 CFU/Litre Legionella pneumophila serogroup 1. The limit of detection (LOD) of the water test is equivalent to 1000 CFU/L when a 100 ml sample is filtered. If smaller or larger volumes are processed the detection limit will be altered accordingly.

Suspended solid content in water samples affects filtration and test performance, including analytical sensitivity. Actual results will vary. Water samples with high levels of suspended solids may block filtration entirely. L. 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 techniques used in other microbiological analysis methods.

The limit of detection of the swab test is 200 CFU/swabbed area.

Test operating limits

The test has been evaluated for operation between 10–45°C (50–113°F). The test has been validated for samples that filter in less than 10 minutes. Samples requiring greater than 10 minutes to filter may give erroneous results. Samples requiring long periods to filter may be indicative of poor system maintenance.

A wide range of non-oxidizing biocides and biodispersants have been checked for cross reaction and interference with the test.

The test should not be used on systems treated with biguanide or tetrakis hydroxymethyl phosphonium sulfate (THPS) based biocides.

Specificity

The test has been shown to be non-reactive with the following bacteria (at $1x10^8$ organisms per sample):

- Acinetobacter calcoaceticus
- Aeromonas hydrophila subsp. Hydrophila
- Bacillus subtilis
- Burkholderia cepacia
- Citrobacter freundii
- Citrobacter koseri
- Enterobacter cloacae
- Escherichia coli
- Klebsiella oxytoca
- Pseudomonas aeruginosa
- Pseudomonas fluorescens
- Pseudomonas putida
- Pseudomonas stutzeri
- Ralstonia pickettii
- Raoultella terrigena
- Streptococcus pyogenes
- Yersinia ruckeri

Organism	≥cfu/mL
L.p Sg-2,3,8,11,13,14	1.00E+08
L.p. Sg-4,5,6,7,9,10,15	1.00E+07
L.p. Sg-12	8.00E+06
S.aureus	2.00E+08

The Lovibond® Legionella Test™ has been shown to produce weak positive results with other legionella pneumophila serogroups at the cfu/mL stated in the above table.

Storage

The test is intended for storage at room temperature 18–22°C (64.4–71.6°F). Do not freeze. When stored correctly, the test will continue to operate within design specification, until the specified expiration date.

Do not use the test or the recovery buffer syringe after the date specified on the packaging of the test. Do not use any test where the foil packaging is perforated.

Disposal

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

Lovibond® Water Testing

Tintometer® Group

Risk Assessment Legionella Test™ EU Kit

Instructions

This kit is designed to test for *Legionella* in water and biofilms in risk areas identified by ECDC* such as:

- Domestic and industrial hot and cold water systems.
- Cooling towers and water tanks.
- Decorative fountains, hot tubs and pools.
- Sinks and showers.
- Misters, sprinklers, air washers, humidifiers and others.

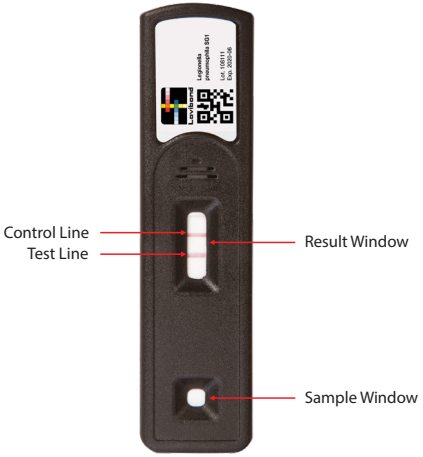
**The European Centre for Disease Prevention and Control*

Risk Assessment Legionella Field Test™ EU Kit product code: 56B006107

Overview

This test is used to detect the presence of Legionella pneumophila serogroup 1 bacteria in water samples from a wide range of sources. The test operates via a Lateral Flow Immunochromatographic Assay (LFICA). Each kit contains the following:

- 4 x individual foil wrapped LFICA tests each with exact volume pipette.
- 2 x hollow fibre filters.
- 2 x syringes containing recovery buffer.
- 2 x swabs.
- 2 x pre-filled vials.
- 2 x 60 ml syringes.
- 1x EU score card to determine action level: score of 1 or greater is = ≥ 1000 CFU/L and score of 6 or greater is 6 or greater = ≥10,000 CFU/L.

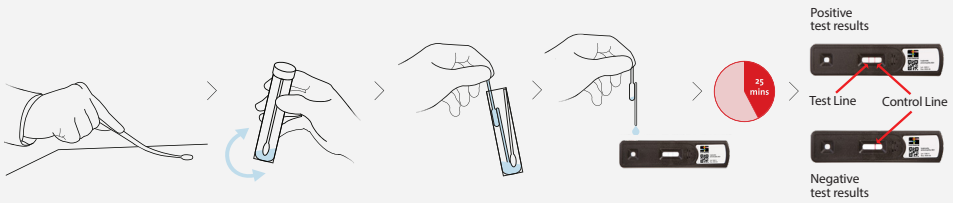


The product is intended for use as part of an overall water treatment, management and risk reduction approach and, as all testing methods including lab culture testing, should NOT be used as the sole method for assessing risks associated with Legionella bacteria.

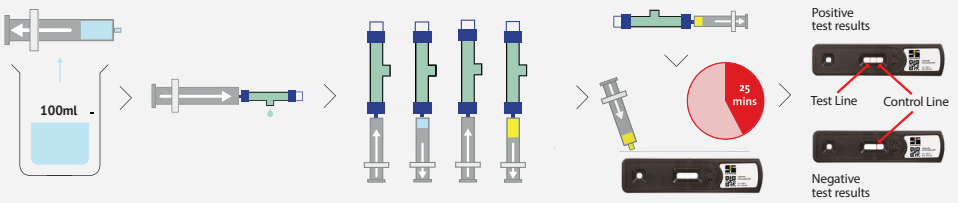
This test is intended for the analysis of water samples. only It is NOT intended for the diagnostic testing, in a clinical or medical situation, of Legionnaires' Disease in humans.

www.lovibond.com

BIOFILM SWAB TESTS



WATER TESTS



For optimum results the test should be performed at room temperature. The foil wrapping should NOT be opened until immediately prior to running the test. If the foil is opened and the test is NOT performed within 60 minutes discard the test.

BIOFILM SWAB TESTS

Step 1. Collect biofilm sample

Identify 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 cm^2. If the surface to be sampled is dry then pre-moisten the swab by dipping it in the pre-filled vial. Wipe the swab across the area to be tested.

Avoid generating aerosols when collecting or handling samples.

Insert the swab into the pre-filled vial and snap off the swab handle.

Step 2. Recover the bacteria

Screw on the lid and shake the tube for at least 20 seconds or until the swab has released the biofilm sample into the recovery buffer.

Step 3. Add sample to the test strip

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

Place the open end of the pipette into the solution in the pre-filled vial, 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 bulb. This is excess and can be ignored. Avoid getting air bubbles in the tube. The pipette filling process may be repeated if necessary to remove air bubbles.

Incorrect use of the pipette can cause flooding of the test (too much sample added) or failure to run (insufficient sample added).

Place the pipette over the small sample window at one end of the strip, and then squeeze the top pipette bulb again. This will dispense the correct amount of sample onto the test strip.

RECORD THE TIME. Allow the test to develop at room temperature for 25 minutes. Leave the test strip sitting on a flat surface during development.

WATER TESTS

Step 1. Take a sample

Collect a water sample of at least 100ml in a clean container.

From the kit, take a 60 ml syringe and draw up 50–60 ml of the sample. Remove the Hollow Fibre Filter from the packaging and tighten the end cap. Next fix the filter onto the luer lock end of the filled 60 ml syringe. Now filter the sample over a sink or other waste water outlet. Repeat this process until all the 100 ml sample has been filtered. This should take no longer than 10 minutes.

Step 2. Recover the bacteria

Disconnect the filter from the 60 ml syringe and discard the syringe. Hold the filter vertically with the cap at the top and the open end pointing towards the floor. Remove the cap and screw it onto the open (opposite) end of the filter (where you just fitted the 60 ml syringe). Now take the small blue capped syringe of recovery buffer, remove the blue cap and attach the syringe to the now open end of the filter with a twist and turn movement.

a) Pull the small syringe plunger back to the **1 ml** mark to re-suspend the recovery buffer, then push the syringe all the way to the 0 ml mark.
b) Repeat this process a further 2 times making 3 in total.
c) Draw the syringe back to collect **0.1 ml** of sample then disconnect from the hollow fibre filter. Avoid creating air bubbles in the syringe. Push and pull the syringe plunger again if necessary to remove any air bubbles.
d) The syringe now contains any recovered bacteria ready for testing.

Step 3. Add sample to test strip

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

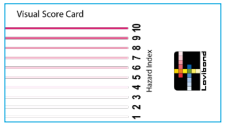
Before use, the test should have two pale blue lines across the result window.

Place the recovery buffer syringe over the small sample window at one end of the test strip. Depress the plunger to dispense the 0.1 ml of recovery buffer, containing any bacteria, onto the test strip.

RECORD THE TIME. Allow the test to develop at room temperature for 25 minutes. Leave the test strip sitting on a flat surface during development.

INTERPRETING THE RESULTS

Leave the test strip sitting on a flat surface during incubation. After 25 minutes, examine the test strip in good lighting. The EU Visual Score Card can be used to read the test accurately. If the test is not read within 35 minutes of adding the water sample, it should be discarded and a new test should be run.



- Score of 1 or greater (on Score Card or App Hazard Index) = ≥ 1000 CFU/L
- Score of 6 or greater = ≥10,000 CFU/L

The test should show one of the following results in the large result window on the test strip:

- Two 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 considered to be a POSITIVE result.

OR

- One RED line across the result window at the end furthest from the sample window. This is a NEGATIVE result.

Positive Results
If a positive result is observed, consult your risk management plan or seek advice from a water management specialist immediately.

Negative Results
A negative result indicates that Legionella pneumophila serogroup 1 was not detected and the concentration was below the detection limit of the test.

Invalid Tests
In the unlikely event that a 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 result is invalid. Repeat the test.

Performance Factors
A positive test result indicates that Legionella pneumophila serogroup 1 was present in the sample above the detection limit. The test does not differentiate between viable and non-viable organisms. The test will detect dangerous viable but non-culturable bacteria, which cannot be detected by traditional laboratory techniques. A positive result does not necessarily mean that viable bacteria are present.

A negative result does not mean that the system is completely free from risks associated with Legionella bacteria.

The test detects Legionella pneumophila serogroup 1. The test does not detect the presence of other Legionella species or serogroups.