Legipid® Legionella Fast Detection

Catalog number:

311-10-01

Package insert

Test based on combined magnetic immunocapture and enzyme-immunoassay (CEIA) for the fast detection of *Legionella spp* in water samples.

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I. INTRODUCTION

Legipid® *Legionella* Fast Detection (Cat. No. 311-10) is a simple and rapid test for the presumptive detection of *Legionella sp* in potable, natural and industrial water. The test combines magnetic immunocapture and enzyme-immunoassay (CEIA) with colorimetric detection for a rapid 1 hour test, following the pre-concentration of a sample.

II. THE Legipid® *Legionella* Fast Detection TECHNOLOGY

Original water sample is concentrated by filtration or similar, and this prepared sample is eluted and dispensed into the test cuvette, to be analyzed by the CEIA method. A suspension of *Legionella* binding magnetic beads is added. *Legionella* cells present in the prepared sample will bind to the antibodies immobilized onto the surface of the beads, to form bacteria/bead complexes. As these complexes can be separated by a magnet, they can be easily washed and resuspended. Next, complexes are incubated with an enzyme-conjugated anti-*Legionella* antibody to form labeled complexes. After washing steps, the complexes are visualized by the colorimetric reaction when enzyme substrates are added. This test includes the following 3 main steps:

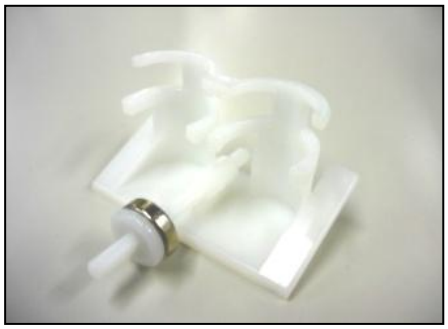
III. KIT REAGENTS AND COMPONENTS

The reference **311-10-01 (10 tests)** contains the elements listed in the following table:

Reagent/component	ID	Quantity provided
Diluent	**L0**	1 bottle (110 mL)
Capture reagent (immunomagnetic particles)	**L1**	10 single-doses (10 x 1 mL)
Washing buffer	**L2**	1 bottle (200 ml)
Enzyme-labeled anti-*Legionella*	**L3**	10 single-doses (10 x 1 mL)
Enzyme co-susbtrates	**L4**	5 tetra dose (5 x 5ml)
Stopping reagent	**L5**	1 bottle(2 mL)
Cuvette	**CB**	10 units
Disposable pipette	**DP**	15 units

The Magnetic Particle concentrator **MP2-Hunter (311-MP2-RA)** contains the elements listed in the following table:

MP2-Hunter (ref. 311-MP2-RA), unit		
Component	**Reference ID**	**Quantity**
Magnetic holder for two cuvettes	311-MP2-SP	1
Cuvette	311-10-CB	2



311-MP2-SP

311-10-CB

Table cloth (311-MP2-TC) could be used to avoid interference on reactions due to excessive proximity between the magnets. If table cloth is not available, then please keep at least 12 cm between magnetic particles hunters.

IV. SHELF LIFE AND STORAGE

Once received, the kit must be stored between +2°C and +8°C, preferably at +4°C. The expiry time of the reagents, properly stored, is 3 months from the manufacturing date. All the reagents are labeled with their own lot number and the storage conditions. These conditions are also displayed on the package. In addition, the protocol includes code, batch number and expiry date, so traceability of all reagents is guaranteed. A certificate of analysis can be requested to the manufacturer.

V. MATERIAL REQUIRED BUT NOT SUPPLIED

- Graduated screw-cap tube, for the filter elution.
- Glass fiber filter, to use as a pre-filter in the filtration system (*).
- Sterile membrane filter, to use with filtration system.
- Container for residue.
- Pipettes of 10-100µl, 100-1000µl and 1-5ml (Optional).
- Filtration device (**), for pre-concentration of water samples by membrane filtration.
- Optional: Primelab (ref. 911-10-PL), colorimeter S2B (ref. 511-10-COL, one unit) and reading cuvettes (ref.511-10-04, 100 units)
- Optional: Vortex apparatus or sonicator, for releasing retained material from the filter (elution can be done manually).

(*)The use of a 2.7 µm-porous size glass fiber filter as pre-filter for water samples is just recommended for the very dirty water samples which filtration maybe difficult.

(**)Note: Contact with Biótica for detailed information on the devices recommended by our technical department

VI. PRECAUTIONS AND RECOMMENDATIONS FOR BEST RESULTS

- This test must be performed by adequately trained personnel.
- This test is designed for the following matrices: potable, natural and industrial water.
- The product is safe under normal use. Avoid contact with eyes. If splashing may occur, wear safety glasses. Avoid contact with skin by wearing gloves. (See MSDS).
- Attention: Certain isolates cannot be detected below 10^6 colony forming units.
- The products are stable and unlikely to react in a hazardous manner under normal conditions of use.
- The product should be disposed of according to local regulations. Dispose of empty containers through the process of recycling or waste disposal.
- The performance of the test depends on strict compliance with the following instructions, especially concerning the correct execution of the protocol:
    - Do not use reagents after their expiry date.
    - Use as negative control the same diluent (L0 reagent) used to prepare the sample (elution)
    - Use a negative control (L0 reagent) for each batch of tests.
    - Leave the reagents at room temperature (18-26 ºC) for at least 30 min before use.
    - Shake reagent L1 before use to ensure homogeneity of immunomagnetic particles.
    - Thoroughly execute the washing steps (L2 reagent).
    - **The cuvettes are disposable. Do not reuse them.**
- Take out of fridge the single-doses and tetra doses necessary to perform scheduled tests
- Use the table cloth (311-MP2-TC). If not, then please keep at least 12 cm between magnetic particles hunters.
- **Reagents are supplied in excess. Do not reuse any leftover amounts of reagents.**

VII. PROTOCOL

It is strongly recommended to read carefully the entire protocol before starting the test.

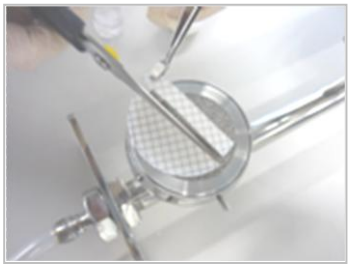
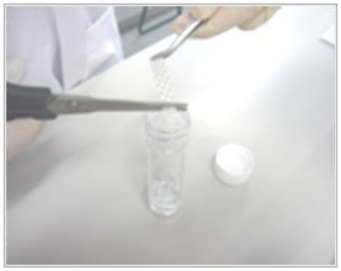
A. Sample preparation

1. Collect the volume of the original water sample to be concentrated (e.g. by filtration).

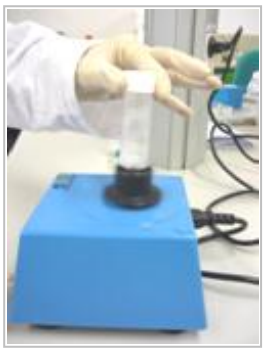
2. Add between 5 and 10 ml, preferably 10 ml, of the diluent reagent in a flask. Use as diluent L0.

3. Filter the collected volume using a polycarbonate filter of 0.40 µm pore diameter or a nitrocellulose filter of 0.45 µm pore diameter. For very dirty samples you can use a glass fiber pre-filter of 2.7 µm pore diameter placed over the filter.

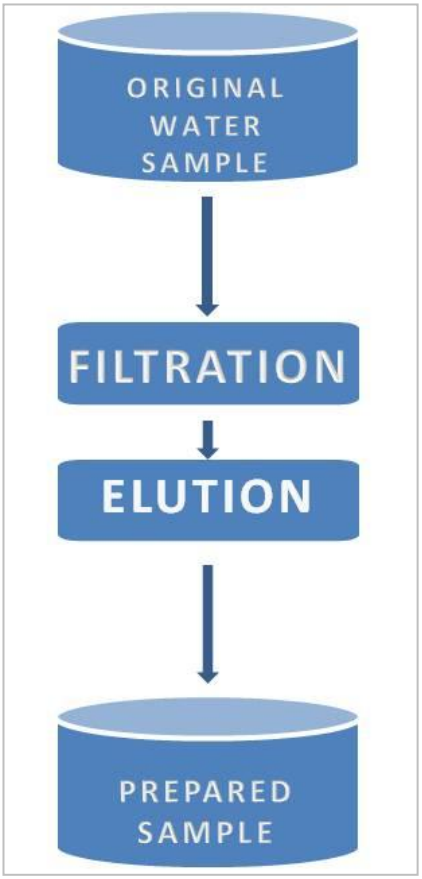

4. Then, carefully separate the filter from the filtration system and deposit it in the flask with the diluent reagent, previously prepared in step 1. Optionally you can use scissors to cut the filter into several pieces. If you have also used the glass fiber pre-filter please remove and discard it.

5. Elute the filter by shaking. The shaking can be:
   a. Manual (2 minutes)
   b. Vortex (2 minutes)
   c. Ultrasound bath (5 minutes)


The eluted sample is called the prepared sample



Note:
With each batch of samples, a negative control must be done using the same diluent reagent (L0)

Protocol based on the ISO 11731 standard for the detection and enumeration of *Legionella* in water.

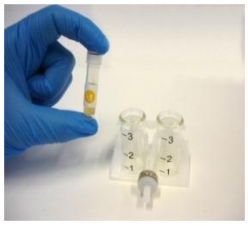
B. Analysis using Legipid® *Legionella* Fast Detection Kit

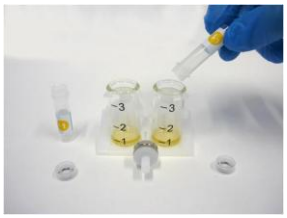
Before starting the test:

- **Make sure the reagents that are going to be used have been left at room temperature for at least 30 minutes.**
- Insert the cuvettes into magnetic particle concentrator MP2.

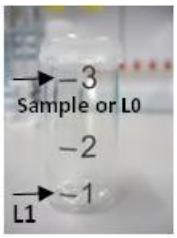
B.1) CAPTURING STEP

1. Separate the magnet. **Shake gently the L1 by repeatedly inverting the single-dose until a completely homogeneous suspension is obtained** and add it completely in the cuvette.

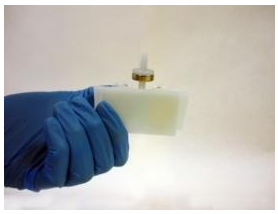
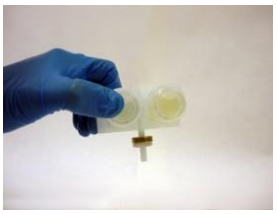




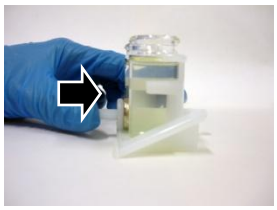
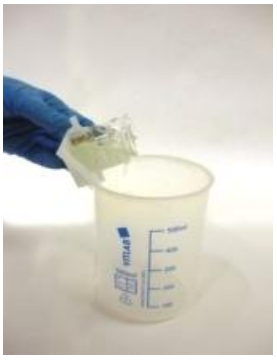
2. Add L0 in the control cuvette (C) **up to line 3** (9ml). Add the sample (previously filtered and eluted) into the test cuvette (T) **up to line 3** (9ml), being careful not to let fall any pieces of filter.



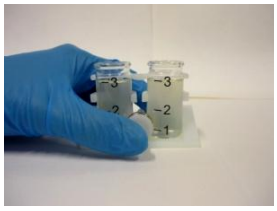

3. With the **LIDS ON**, shake **gently** by inverting the cuvettes **3 times every 3 minutes, for 15 minutes**.

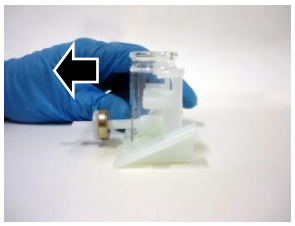
4. REMOVE THE LIDS AND DISCARD THEM. Fix the magnet to the cuvettes and **wait 5 minutes** for the immuno-magnetic particles to be retained.

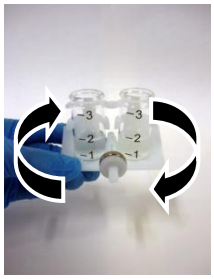



5. Empty the cuvettes from the opposite side to the magnet, holding the magnet against the cuvettes so that the retained particles are not lost.

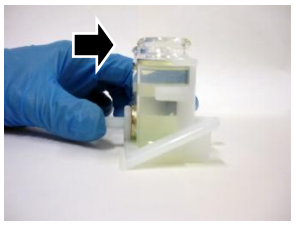


6. Separate the magnet from the cuvettes and add the reagent L2 up to **line 2** (4.5 ml) in each cuvette. Shake **vigorously** WITHOUT LIDS until the particles are resuspended **(10 seconds)**.

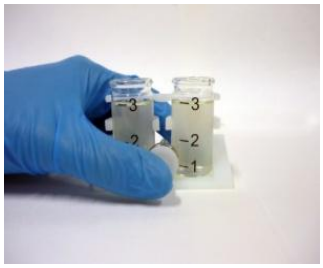




7. Fix the magnet to the cuvettes and wait **3 minutes** for the immunomagnetic particles to be retained.



8. Empty the cuvettes from the opposite side to the magnet, holding the magnet against the cuvettes so that the retained particles are not lost.



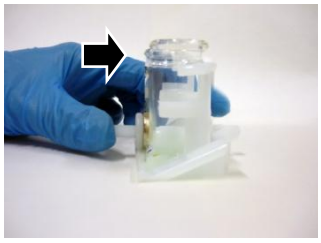

B.2) MARKING STEP

1. Separate the magnet. Add a single-dose of reagent **L3** per cuvette and shake **vigorously** for **10 seconds** until the particles are resuspended.

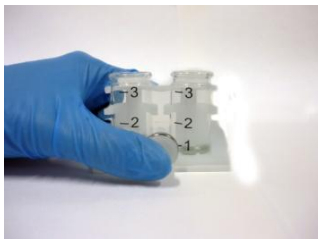


2. Shake **gently** WITHOUT LIDS **every 2 minutes for 10 minutes.**

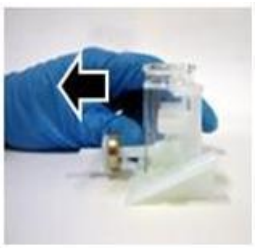
3. Fix the magnet to the cuvettes and **wait 3 minutes** for the immunomagnetic particles to be retained.

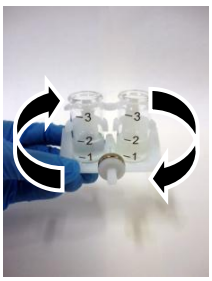


4. Empty the cuvettes **from the opposite side to the magnet**, holding the magnet against the cuvettes so that the retained particles are not lost.

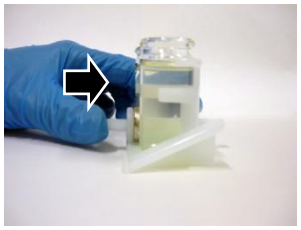
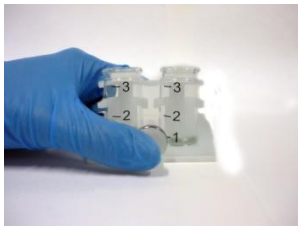



5. Separate the magnet from the cuvettes and add the L2 reagent **up to line 2** (4.5 ml) in each cuvette. Shake **vigorously** WITHOUT LIDS until the particles are resuspended **(10 seconds)**.





6. Fix the magnet to the cuvettes and wait **3 minutes** for the immunomagnetic particles to be retained.

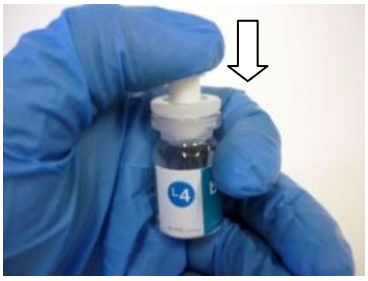



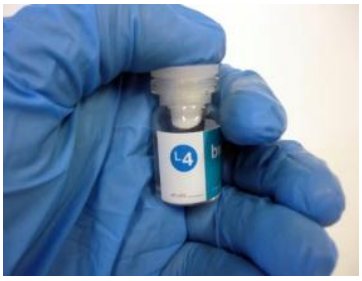
7. ***Repeat points 4, 5, &6 (of this section B.2 MARKING STEP) two more times***

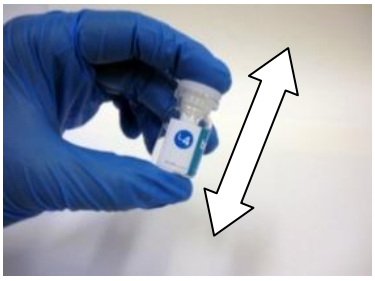
B.3) DETECTION STEP

1. Empty the cuvettes from the **opposite side to the magnet**, holding the magnet against the cuvettes so that the retained particles are not lost.

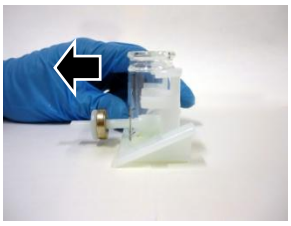
2. Prepare reagent L4 (one vial for 4 tests): break the seal. Remove the plastic protector and push the plunger cap all the way down. **Shake vigorously the mixture for 10 seconds**. **Once L4 is prepared, the mixture should be used immediately.**

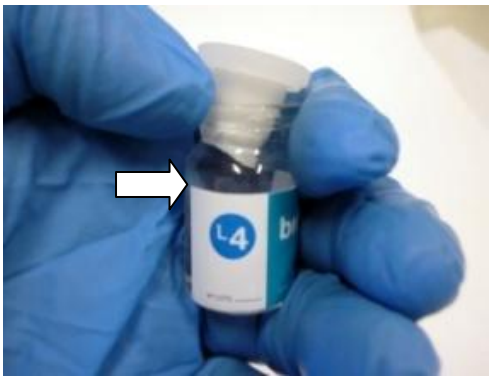


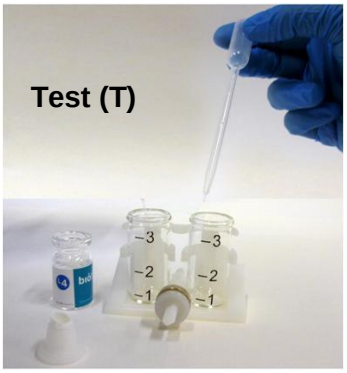




3. Separate the magnet. Open the homogenized vial of L4 by removing the cap just before using, applying slight lateral pressure. **Immediately add the L4 up to line 1 (1 ml)** to each cuvette using disposable pipette.



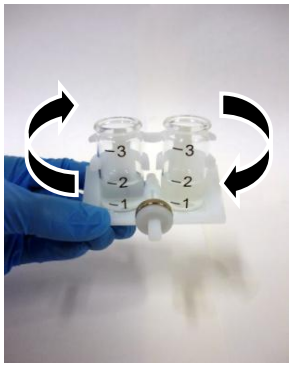




4. Shake **gently** WITHOUT LIDS **(2 minutes).** Begin shaking **vigorously** for the first **10 seconds** until the particles are resuspended.

- If *Legionella* concentration is **visually** estimated, then go to **Sub-Protocol A**.

- If *Legionella* concentration is estimated by **measuring** the **absorbance** at 429nm, then go to **Sub-Protocol B**.

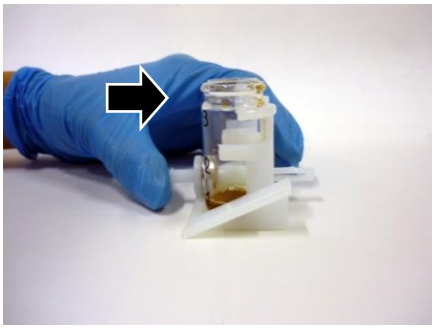


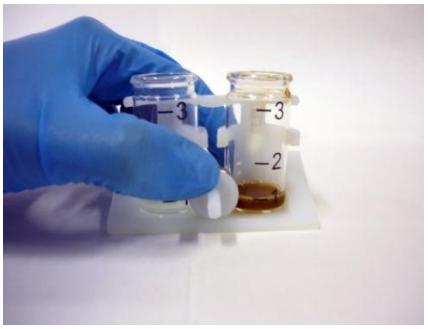
Sub-Protocol A

5. After these 2 **minutes**, if color difference appears between test (T) and control (C), then go to next step. If not, let color develop, shaking **gently** for 8 **more minutes (10 minutes in total)** before the next step.

6. Add **3 drops of L5 (100 µl)** and shake **gently** WITHOUT LIDS for 5 seconds.

7. Fix the magnet to the cuvettes to capture the particles and wait for **5 minutes.**





8. See C. INTERPRETATION AND TEST RESULT REPORT- **paragraph C.1.,** on page 10.

Sub-Protocol B

5. Add **3 drops (100 µl) of L5** both in the control cuvette (C) and test cuvette(s) (T) and shake **gently** MP2 for 5 seconds.

6. Fix the magnet to the cuvettes to retain the magnetic particles and wait for **5 min.**

7. Place the supernatants of both the control (C) and the test (T) cuvette(s) into corresponding reading cells.
Important note: Pipet the supernatant from the opposite side to the magnet, taking care not to drag the particles retained by the magnet.

8. Measure the absorbance at 429 nm on a cell filled with distilled water. Adjust absorbance to zero.

9. Measure the absorbance at 429 nm of the supernatant of the control (C) as a reference. Then adjust absorbance to zero.

10. Measure the absorbance of each test (T) supernatant. Read immediately; always within 10 minutes after the end of the colorimetric reaction.

11. See C. INTERPRETATION AND TEST RESULT REPORT- **paragraph C.2**., on page 11.

Note: If the path length of the measuring cell is not 1 cm, path length correction is needed. Please follow the instructions of the optical reader's manufacturer.

C. INTERPRETATION AND TEST RESULT REPORT

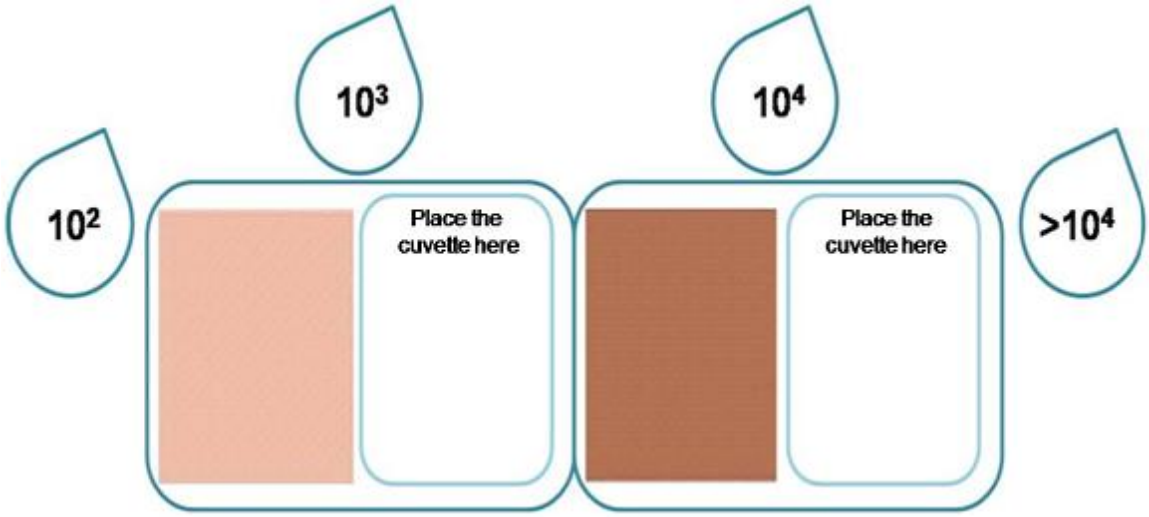
C.1. Visual interpretation

A test (T) is considered POSITIVE if:

1. The test (T) has higher color than the control (C) 2 **minutes** after the beginning of the colorimetric reaction. In such case, stop the reaction following the instructions of this package insert. The general estimation of the level of *Legionella* can be obtained by comparing the test color (T) **with the color chart**.

Color chart To estimate the level of *Legionella sp* in a positive sample **2 minutes** after the beginning of the color reaction, place the test (T) cuvette next to the following color chart.

Results in CFU / volume examined



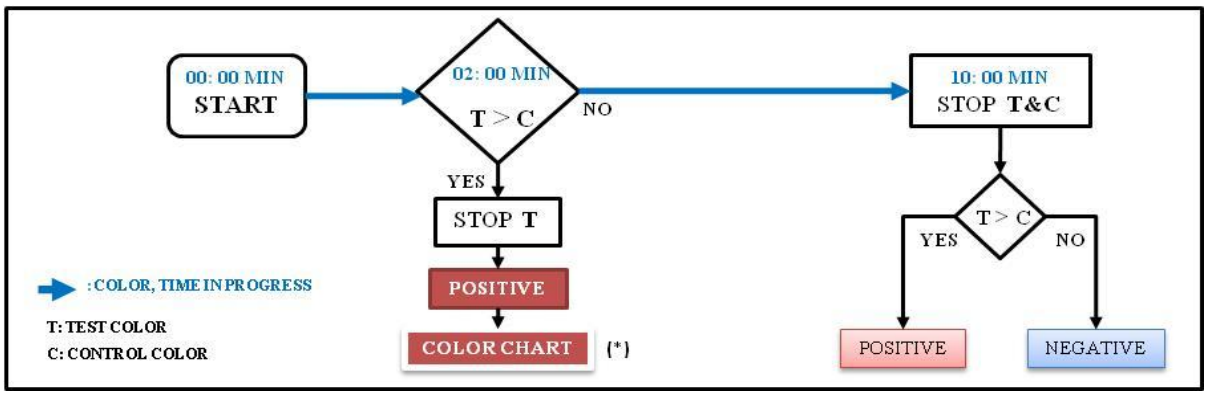
For weaker colors than the first color of the chart, the estimated level of *Legionella sp* is up to two orders of magnitude (10^2 CFU/volume examined). Below the second color of the chart, the estimated level of *Legionella sp* is up to three orders of magnitude (10^3 CFU/volume examined). For stronger colors than the second color of the chart, the estimated level of *Legionella sp* is equal or greater than four orders of magnitude (10^4 CFU/volume examined).

2. If there is no color difference 2 minutes after the beginning of the reaction, let the reaction progress for 8 **more minutes (10 minutes in total)**. A positive test (T) for *Legionella* must have a stronger color than the control (C) color 10 minutes after the beginning of colorimetric reaction. The estimated level of *Legionella* is low, up to two orders of magnitude (10^2 CFU/volume examined).

A test (T) is considered NEGATIVE if:

3. The test (T) doesn't have color difference with the control (C) **10 minutes** after the beginning of the colorimetric reaction.

Interpretation of test results is summarized in the following diagram:



(*) Level of Legionella can be estimated by the use of the color chart

REMEMBER: **Don't stop the control (C) at 2 min, unless all conducted samples are positive at 2 min.** Discard the cuvettes at the end of the test. Do not reuse cuvettes or any leftover reagents.

C.2. Optical reading

(a) *Control value* — After adjusting the absorbance to zero with distilled water, measure the absorbance of the control (C). Adjust the absorbance to zero with the control (C) before measuring the absorbance of the test (T) samples.

(b) *Cutoff value* — This value of relative absorbance is A_r=0.04 units.

(c) *Negative results* — Test (T) supernatants with relative absorbance readings lower than the cutoff value are negative and are reported as Not Detected.

(d) *Positive results* — Test (T) supernatants with relative absorbance readings equal to or greater than the cutoff value are positive and are reported as Detected.

(e) For the positive results, perform $\log_{10}$ transformation of the relative absorbance.

(f) Estimate the concentration of the target in the volume examined by introducing the $\log_{10}$ value of the relative absorbance (A_r) into the following equation:

$y = 2.3061\ x + 4.9815$, where $x = \log_{10}(A_r)$ and $y = \log_{10}$ (CFU/volume examined)

(g) Result can be finally obtained by doing the inverse log transformation:

Contamination of the target (CFU/volume examined) = 10^y

VIII. CONFIRMATION OF POSITIVE RESULTS

For the AOAC-RI certification purpose, a positive Legipid® *Legionella* Fast Detection result was considered presumptive positive and it was confirmed by standardized culture methods (e.g. ISO 11731:1998).

It is possible to store a 0.1-0.5 ml volume of the prepared sample before carrying out the confirmations. In the event of results that are not in agreement between Legipid® *Legionella* Fast Detection and the confirmation method, the user should follow the necessary steps to ensure the validity of the results. Positive deviation can be associated with the poor target recovery by culture (viable but non culturable-VBNC- bacteria, microbiota that inhibits *Legionella* growth, etc), or insufficient compliance with the washing in the marking step of the test protocol.

IX. TEST PERFORMANCES AND VALIDATIONS

The Legipid® *Legionella* Fast Detection kit is a rapid and simple test for the detection of *Legionella sp* in water samples. This kit has a relative level of detection of 93 CFU/volume examined (LOD50). With optical reading this kit has a limit of detection of 40 CFU/volume examined and a limit of quantification of 60 CFU/volume examined.

PERFORMANCE TESTED AOAC RESEARCH INSTITUTE LICENSE NUMBER 111101

Legipid® *Legionella* Fast Detection kit is validated by the AOAC-Research Institute under the Performance Tested Method Program for potable, natural and industrial water. Certificate no. 111101.

X. REFERENCES

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Notice to purchaser: Use this product only for environmental testing

India Contact:

Life Technologies (India) Pvt. Ltd.

306, Aggarwal City Mall, Road No. 44, Pitampura, Delhi – 110034, India

Mobile: +91-98105-21400, Tel: +91-11-42208000, 8111, 8222, Fax: +91-11-42208444

Email: customerservice@lifetechindia.com, www.atzlabs.com ; www.lifetechindia.com