EN

Legipid® Legionella Fast Detection

Catalog number:

311-10-04

Package insert

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

biotica®

FAST DETECTION FOR LIFE

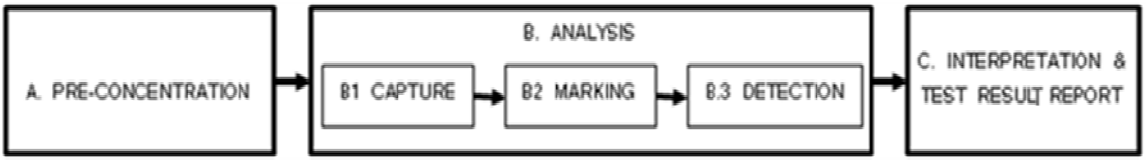
TABLE OF CONTENTS

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 **311-10-04 (100 tests)** contains the elements listed in the following table:

Reagent/component	ID	Quantity provided
Diluent	**L0**	1 bottle (1050 mL)
Capture reagent (immunomagnetic particles)	**L1**	1 bottle (105 mL)
Washing buffer	**L2**	2 bottle (2 X 940 mL)
Enzyme-labeled anti-*Legionella*	**L3**	1 bottle (105 mL)
Enzyme co-susbtrates	**L4**	35 tetra dose (35 x 5 ml)
Stopping reagent	**L5**	1 bottle (11 mL)
Cuvette	**CB**	100 units

Magnetic Particle Concentrators contain the elements listed in the following table:

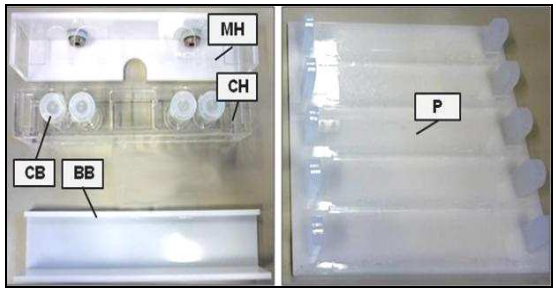
MP2-Hunter (ref. 311-MP2-RA), unit		
Component	**Reference ID**	**Quantity**
Magnetic holder for two cuvettes	311-MP2-SP	1
Cuvette	311-10-CB	2
MP4-Hunter (ref. 311-MP4-00), unit		
Component	**Reference ID**	**Quantity**
Magnetic holder for four cuvettes	311-MP4-MH	7
Insertable holder for four cuvettes	311-MP4-CH	7
Box bracket	311-MP4-BB	7
Cuvette	311-10-CB	20
Table Cloth(*)	311-MP4-TC	1
Platform	311-MP4-P	1
Orbital Shaker	311-MP4-AGT	1

(*) Use it 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 concentrators. Table cloth (311-MP2-TC) for the MP2-Hunter is independently ordered.

311-MP2-SP

311-10-CB

311-MP4-CB



MP2-Hunter recommended for ≤ 10 tests per batch, and MP4-Hunter for >10 tests per batch.

IV. SHELF LIFE AND STORAGE

Once received, the kit must be stored between +2℃ and +8℃, preferably at +4℃. The expiry time of the reagents, properly stored, is 6 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.
- Filtration device (**), for pre-concentration of water samples by membrane filtration.
- Optional: 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).

(*)We strongly recommend using a 2.7 µm-porous size glass fiber filter as pre-filter for water samples, especially when filtration is 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 or Ringer 1/40) used in the sample preparation (elution).
    - Use a negative control (L0 reagent or Ringer 1/40) 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**.
- The following volume of each reagent (per test to be performed) should be left at room temperature at least 30 minutes before use:
    - L0: 10 ml per test.
    - L1: 1 ml per test (first, shake gently the L1-bottle until obtaining a completely homogeneous suspension).
    - L2: 18 ml per test.
    - L3: 1 ml per test
    - L4: each bottle is for 4 tests
    - L5: 0.1 ml per test.
- Use the table cloth (311-MP2-TC, 311-MP4-TC) corresponding to the magnetic particle concentrator used.
- **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 or Ringer 1/40 solution.

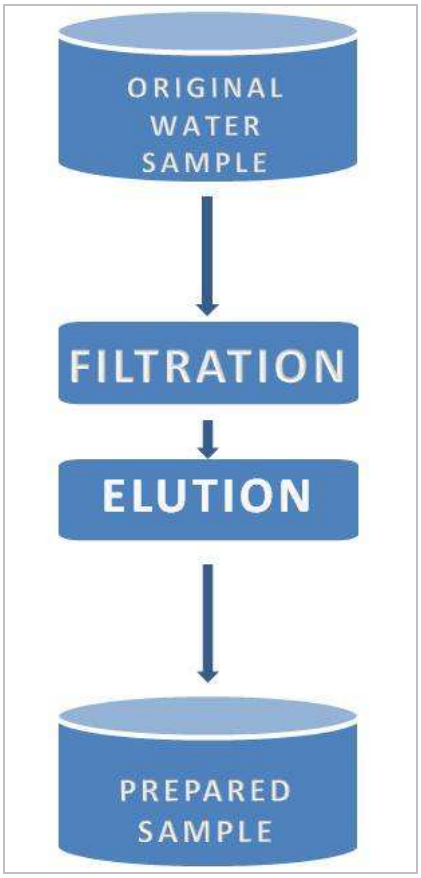
3. Filter the collected volume using a glass fiber pre-filter of 2.7 μm pore diameter placed over a nitrocellulose filter of 0.45 μm pore diameter or over a polycarbonate filter of 0.40 μm pore diameter.


4. Remove the glass fiber pre-filter and discard it. 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.


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 or Ringer 1/40 solution)

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

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

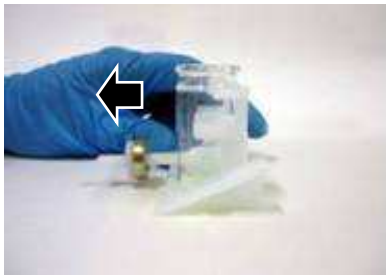
B.1 User of MAGNETIC PARTICLE CONCENTRATOR FOR TWO CUVETTES (MP2-HUNTER)

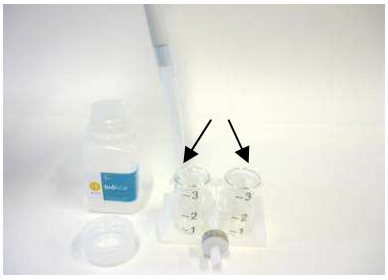
Before starting the test:

- **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.

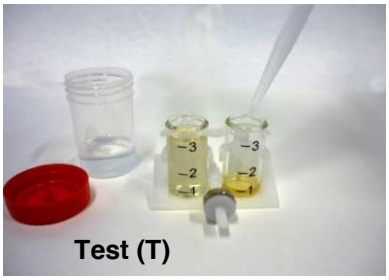
B.1.1) CAPTURING STEP

1. Separate the magnet. **Shake gently the L1 by repeatedly inverting the bottle until a completely homogeneous suspension is obtained** and add **1ml** in each cuvette.

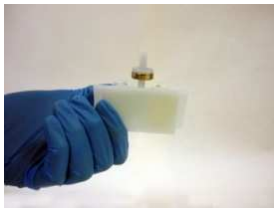


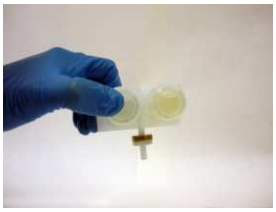


2. Add **9ml** of L0 (or Ringer 1/40) in the control cuvette (C). Add **9 ml** of the sample (previously filtered and eluted) into the test cuvette (T), being careful not to let fall any pieces of filter.

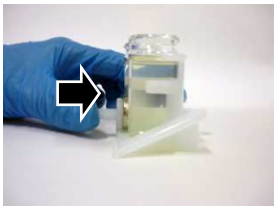


3. With the **LIDS ON**, shake **gently** by inverting the cuvettes **3 times every 3 minutes, for 15 minutes**.

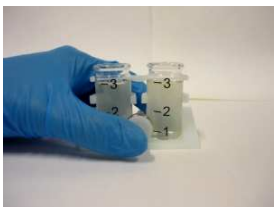




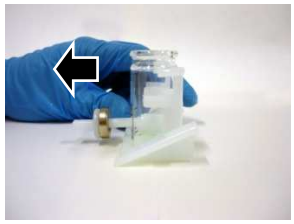
4. REMOVE THE LIDS AND DISCARD THEM. Fix the magnet to the cuvettes and **wait 5 minutes** for the immuno - magnetic particles to be retained.

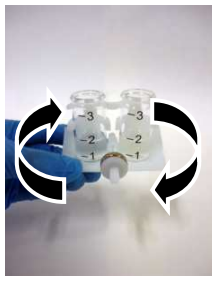


5. Empty the cuvettes from the opposite side to the magnet, holding the magnet against the cuvettes so that the retained particles are not lost.

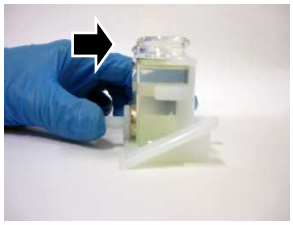


6. Separate the magnet from the cuvettes and add **4.5 ml** of reagent L2.Shake **vigorously** WITHOUT LIDS until the particles are resuspended **(10 seconds)**.

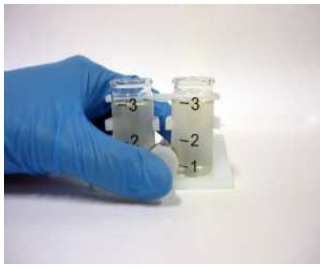




7. Fix the magnet to the cuvettes and wait **3 minutes** for the immunomagnetic particles to be retained.

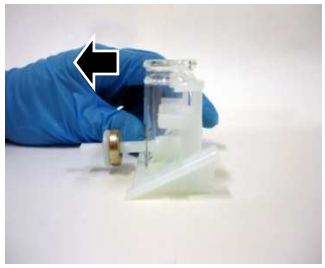


8. Empty the cuvettes from the **opposite side to the magnet,** holding the magnet against the cuvettes so that the retained particles are not lost.



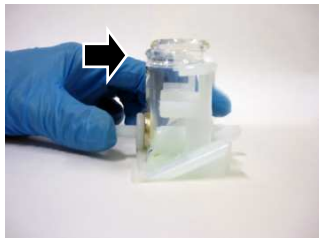

B.1.2) MARKING STEP

1. Separate the magnet. Add **1 ml of L3** per cuvette and shake **vigorously** for **10 seconds** until the particles are resuspended.

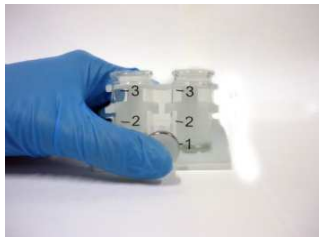



2. Shake **gently** WITHOUT LIDS **every 2 minutes for 10 minutes**.

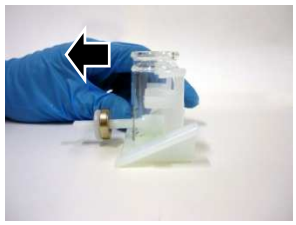
3. Fix the magnet to the cuvettes and **wait 3 minutes** for the immunomagnetic particles to be retained.

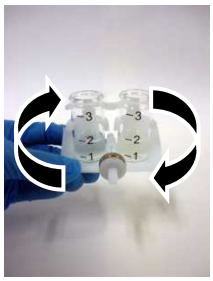


4. Empty the cuvettes **from the opposite side to the magnet**, holding the magnet against the cuvettes so that the retained particles are not lost.

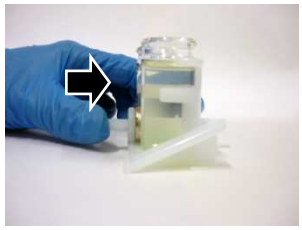
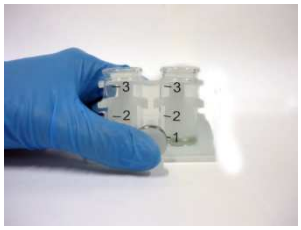



5. Separate the magnet from the cuvettes and add **4.5 ml** of reagent L2.Shake **vigorously** WITHOUT LIDS until the particles are resuspended **(10 seconds)**.





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

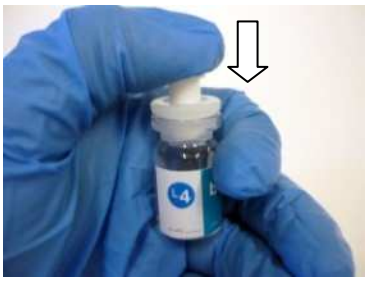



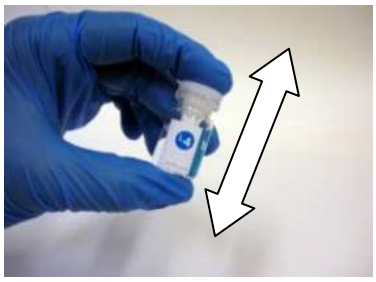
7. ***Repeat points 4, 5, &6 (of this section B.1.2 MARKING STEP) two more times***

B.1.3) DETECTION STEP

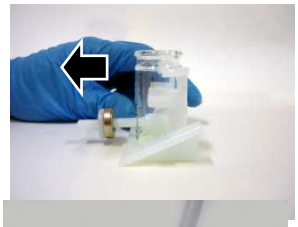
1. Empty the cuvettes from the **opposite side to the magnet**, holding the magnet against the cuvettes so that the retained particles are not lost.

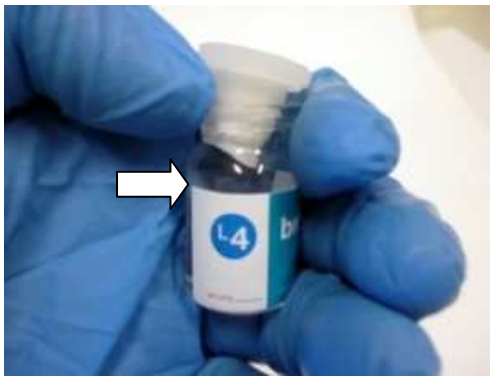
2. Prepare reagent L4 (one vial for 4 tests): break the seal. Remove the plastic protector and push the plunger cap all the way down. Shake **vigorously** the mixture for **10 seconds**. **Once L4 is prepared, the mixture should be used immediately.**





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

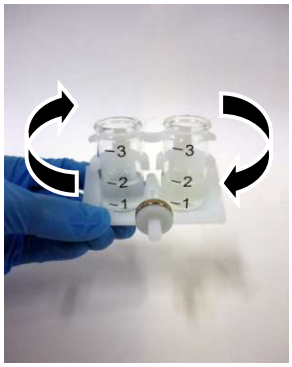




4. Shake **vigorously** WITHOUT LIDS **(10 seconds),** until the particles are resuspended. Then, shake **gently** for 2 **minutes**.

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

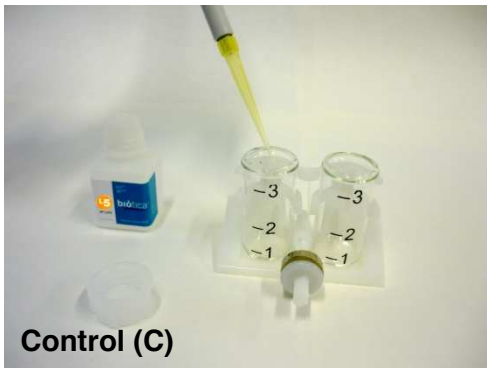
- If *Legionella* concentration is estimated by **measuring** the **absorbance** at 429nm, then go to **Sub-Protocol B**.

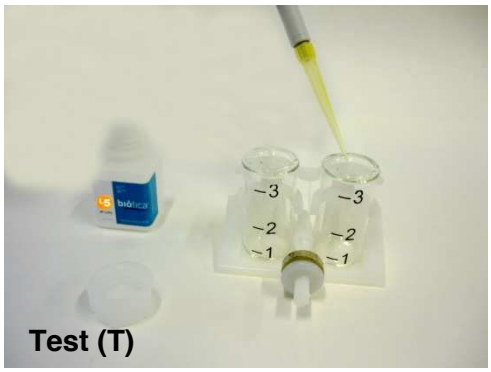


Sub-Protocol A

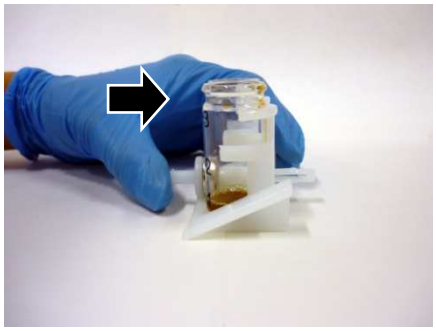
5. After these 2 minute, if color difference appears between test (T) and control (C), then go to next step. If not, let color develop, shaking **gently** for 8 **more minutes (10 minutes in total)** before the next step.

6. Add **100 µl of L5** and shake **gently** WITHOUT LID 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 15.

Sub-Protocol B

5. Add **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 16.

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.

B.2 User of MAGNETIC PARTICLE CONCENTRATOR FOR FOUR CUVETTES (MP4-HUNTER)

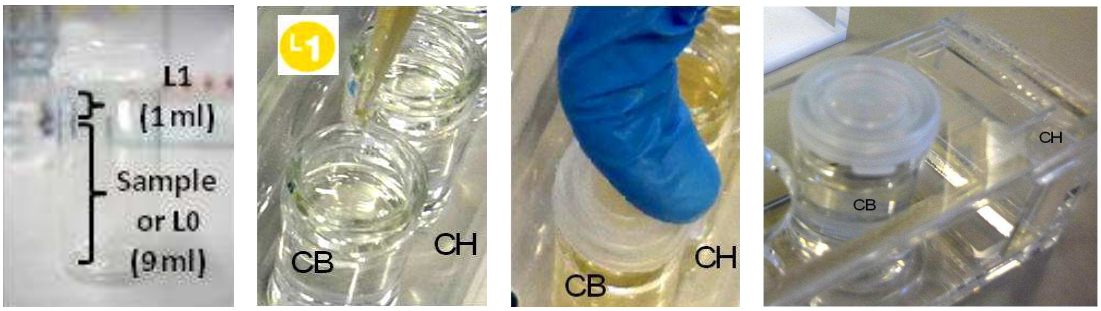
Before starting the test:

- **Make sure the reagents that are going to be used have been left at room temperature for at least 30 minutes.**
- Insert the cuvettes (CB) into their holder (CH), as many cuvettes as tests to be done.
- Put platform (P) on the orbital shaker

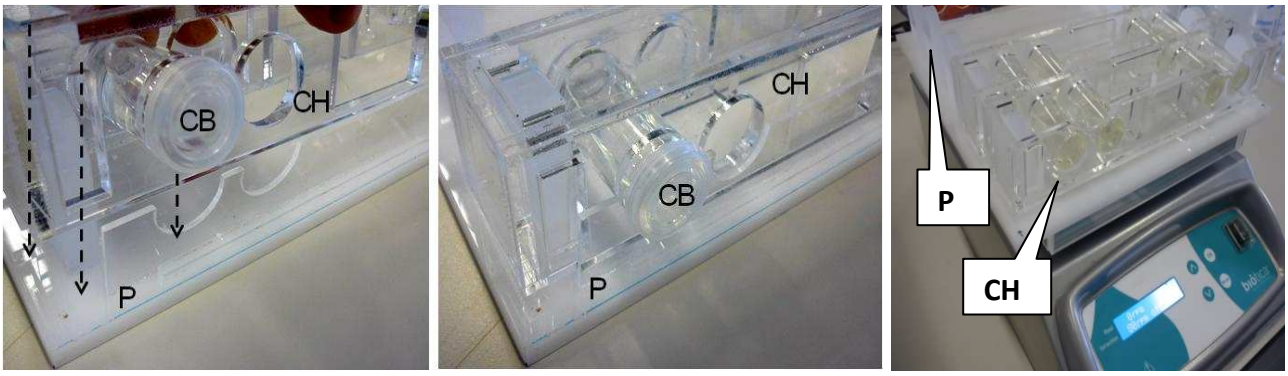
B.2.1) CAPTURING STEP

1. First, add each **prepared sample (9ml)** into its corresponding cuvette (if it is necessary complete the volume up to 9ml with L0 or Ringer 1/40). Then add L0 or Ringer 1/40 **(9ml)** into the control cuvette (one control per batch). Use the same diluent (L0 or Ringer 1/40) for both control and test cuvettes.

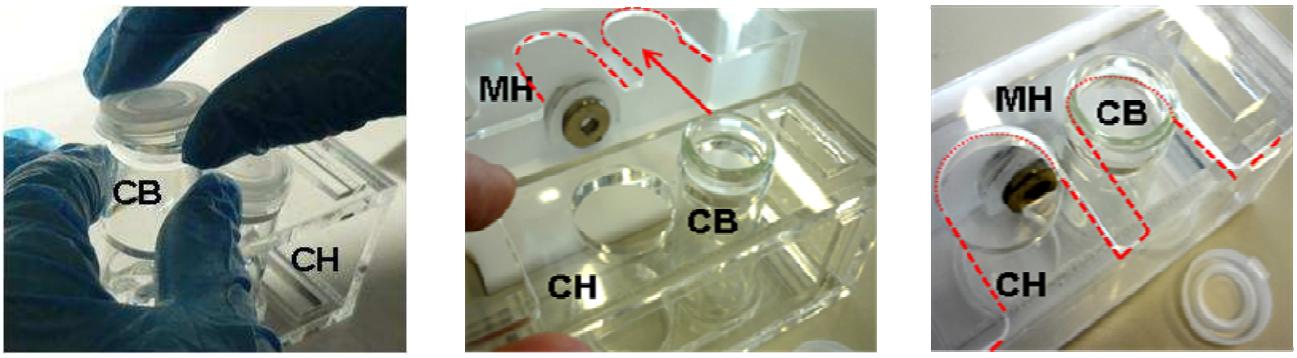
2. Shake gently the L1 by repeatedly inverting the bottle until a completely homogeneous suspension is obtained. Resuspend by repeated pipetting and then add **1 ml** to each cuvette. Put the LIDS ON each cuvette.

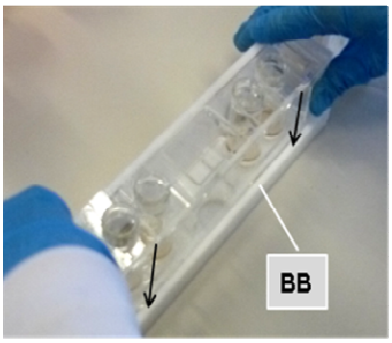


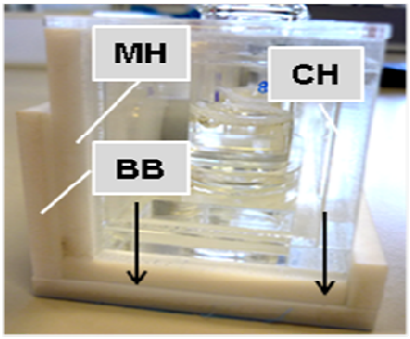
3. Place the cuvette holder (CH) module into the platform (P) so that the cuvettes **are positioned horizontally**. Then shake in the orbital shaker at **80 rpm** for **15 minutes**.

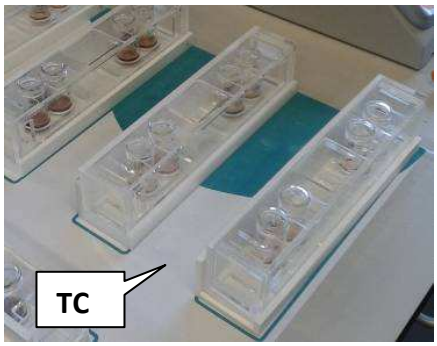


4. Remove each cuvette holder (CH) from the platform (P) and REMOVE AND DISCARD THE LID from each cuvette (in vertical position). Insert each cuvette holder (CH) into a magnetic holder (MH). Fix the assembly into the box bracket (BB) (push and down). Place the modules in the table cloths (TC) and wait **for 5 minutes** for the particles to be retained.



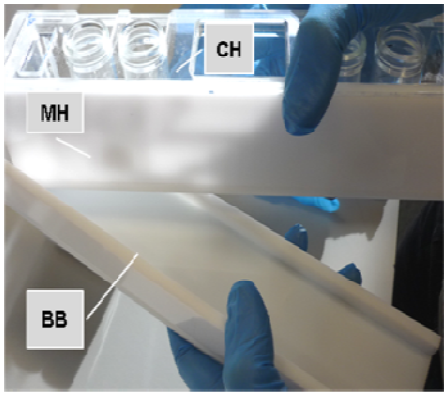


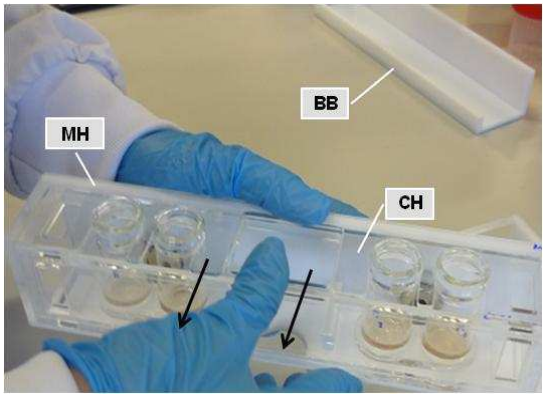


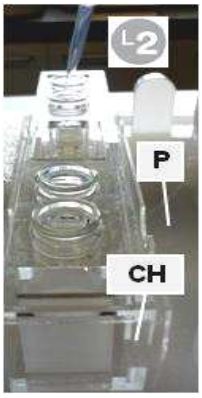


5. Discard the supernatant by emptying the cuvettes from the **opposite side to the magnet**, rotating the assembly.

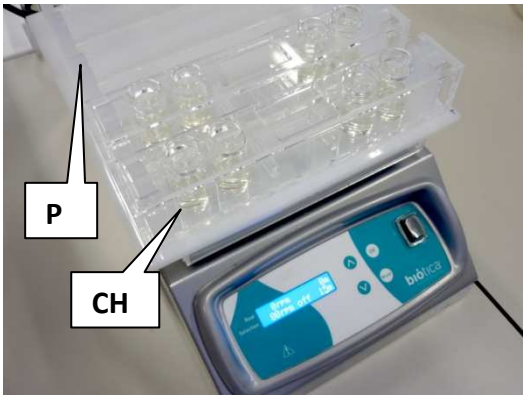
6. Remove the box bracket (BB). Separate the cuvette holder (CH) from the magnetic holder (MH) and place the cuvette holder (CH) in vertical position on the platform (P) in the orbital shaker. Then add **4.5 ml** of the L2 reagent into each cuvette.







7. Shake at **350 rpm** WITHOUT LIDS for **10 seconds**, until the particles are resuspended.

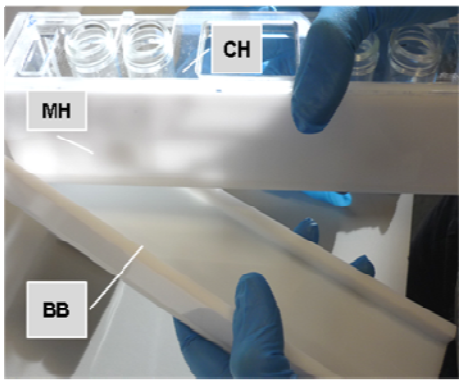


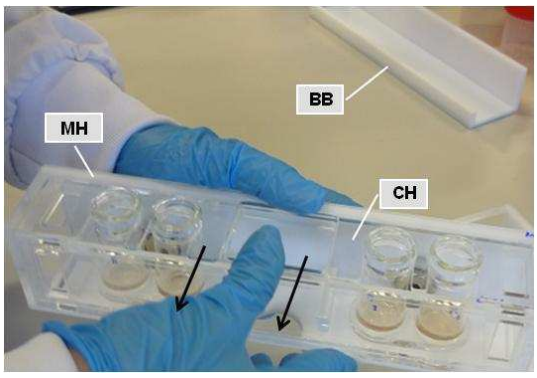
8. Remove the cuvette holder (CH) from the platform (P). Insert the cuvette holder into the magnetic holder (MH) and then into the box bracket (BB) (push and down). Place the modules in the table cloth (TC) and wait **3 minutes** for the particles to be retained.

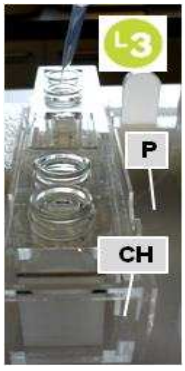
9. Discard the supernatant by emptying the cuvettes from the opposite side to the magnet, rotating the assembly.

B.2.2) MARKING STEP

1. Remove the box bracket (BB). Separate the cuvette holder (CH) from the magnetic holder (MH) and place the cuvette holder (CH) in vertical position on the platform (P) and on the orbital shaker. Add **1 ml** of the L3 reagent to each cuvette.







2. Shake WITHOUT LIDS at **250 rpm** for **10 seconds** and then at **80 rpm** for **10 minutes**.

3. Remove each cuvette holder (CH) from the platform (P). Insert each cuvette holder (CH) into a magnetic holder (MH) and insert each assembly into a box bracket (BB). Wait for **3 minutes** for the magnetic particles to be retained.

4. Discard the supernatant by emptying the cuvettes from the **opposite side to the magnet**, rotating the assembly.

5. Remove the box bracket (BB). Separate each cuvette holder (CH) from its magnetic holder (MH) and place the cuvette holders (CH) in vertical position on the platform (P) and on the orbital shaker. Then add **4.5 ml of the L2 reagent to each cuvette**.

6. Shake at **350 rpm** for **10 seconds** WITHOUT LIDS, until the particles are resuspended.

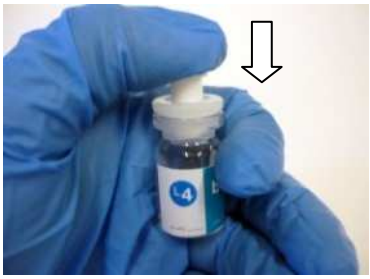
7. Remove each cuvette holder (CH) from the platform (P). Insert each cuvette holder (CH) into a magnetic holder (MH) and insert each assembly into a box bracket (BB). Wait for **3 minutes** for the magnetic particles to be retained.

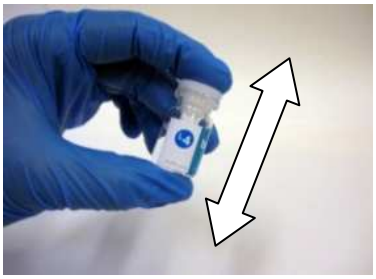
8. ***Repeat points 4, 5, 6 & 7 of this section B.2.2) MARKING STEP two more times.***

B.2.3) DETECTION STEP

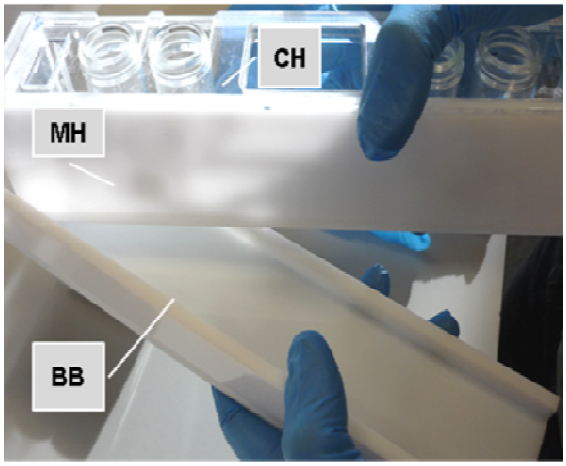
1. Empty the cuvettes from the opposite side to the magnet, rotating the assembly.

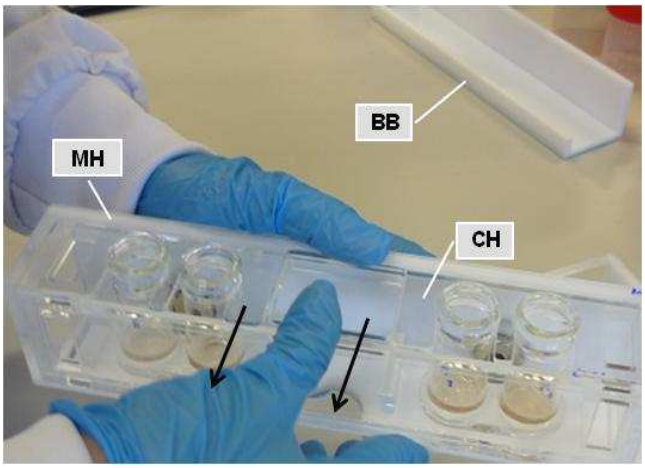
2. Prepare reagent L4 (one vial per 4 tests): break the seal. Remove the plastic protector and push the plunger cap all the way down. Shake **vigorously** the mixture for **10 seconds**. **Once L4 is prepared, the mixture should be used immediately.**

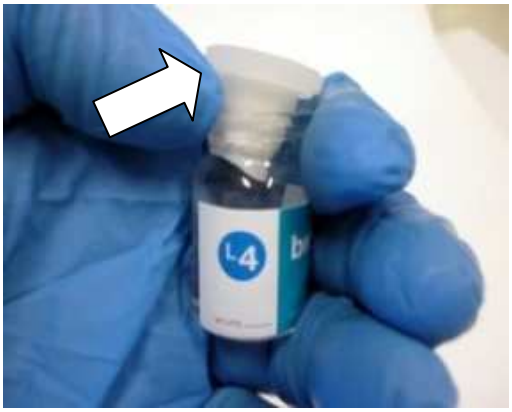




3. Remove the box bracket (BB). Separate each cuvette holder (CH) from its magnetic holder (MH) and place the cuvette holder (CH) in vertical position on the platform (P) and on the orbital shaker. Open the homogenized vial of L4 by removing the cap just before using, applying slight lateral pressure. **Immediately add 1 ml to each cuvette using pipette**.







4. Shake WITHOUT LIDS at **250 rpm** for **10 seconds** and then at **80 rpm** for 2 **minutes**.

- 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. Stop the shaker. Pipette **100 µl** of L5 reagent only into those samples whose color (T) is higher than the control color(C). Transfer only these cuvettes from the platform (P) to the **auxiliary cuvette holder** (CH) and then go to **next step**. For the samples whose test color (T) did not differ from control color (C), let color develop **(shaking again at 80 rpm)** for 8 **more minutes (10 minutes in total)** and then go to **step 8**.

REMEMBER: **Don´t stop control (C) if you have tests**

Which need 8 more minutes of reaction

6. Insert each **auxiliary cuvette holder** (CH) into a magnetic holder (MH) and the assembly into a box bracket (BB). Wait for **5 minutes** for the particles to be retained.

7. Compare the test color (T) **with the color chart**. See C. INTERPRETATION AND TEST RESULT REPORT on page 15, **paragraph C.1, point 1**.

8. Stop the shaker after **10 minutes** of colorimetric reaction.Pipette **100 µl** of L5 reagent both in the samples and in the control (C), and shake at **80 rpm** for **5 seconds** WITHOUT LIDS.

9. Remove the cuvette holder (CH) from the platform (P). Insert into a magnetic holder (MH) and then into a box bracket (BB). Wait **5 minutes** for the particles to be retained.

10. Compare the test color (T) **with the control color (C)**. See C. INTERPRETATION AND TEST RESULT REPORT on page 15, **paragraph C.1, points 2 and 3**.

Sub-Protocol B

5. Stop the shaker. Add **100 µl of L5** both in the control cuvette (C) and test cuvette(s) (T) and shake for **5 seconds at 80 rpm**.

6. Remove each cuvette holder (CH) from the platform (P). Insert each cuvette holder (CH) into a magnetic holder (MH) and insert each assembly into a box bracket (BB). Wait **5 minutes** for the magnetic particles to be retained.

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 16.

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

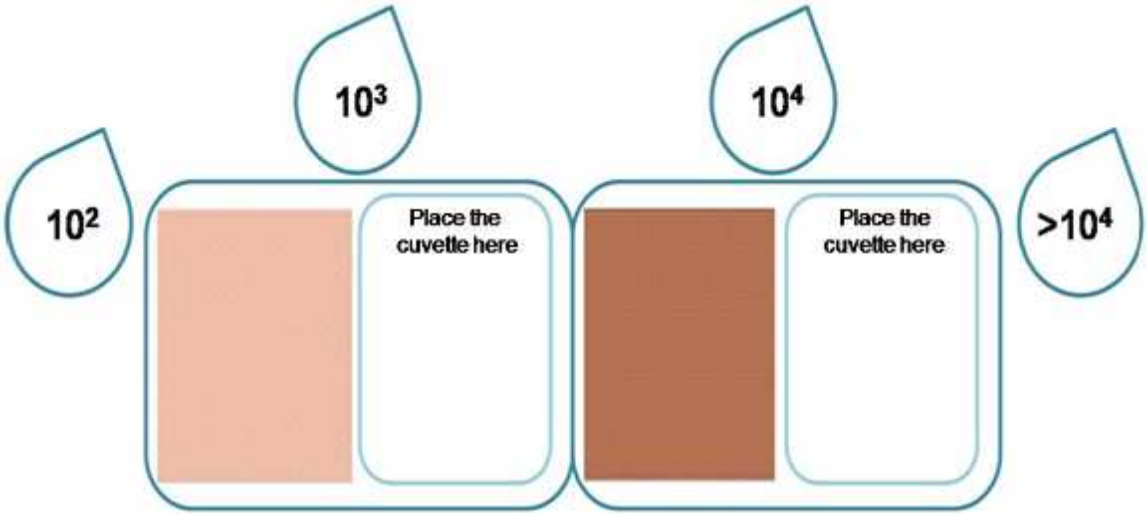
C.1. Visual interpretation

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



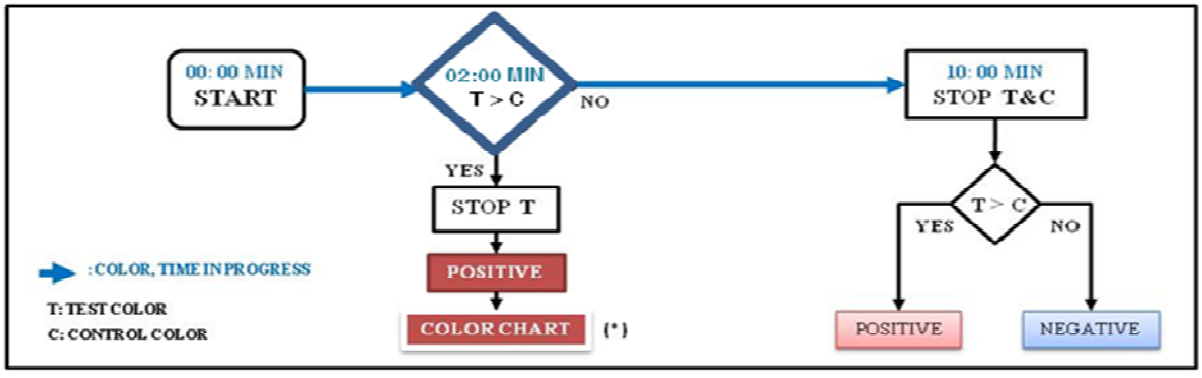
For weaker colors than the first color of the chart, the estimated level of *Legionella sp* is up to two orders of magnitude (10^2 CFU/volume examined). Below the second color of the chart, the estimated level of *Legionella sp* is up to three orders of magnitude (10^3 CFU/volume examined). For stronger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

In the context of AOAC-RI certification, a positive Legipid® *Legionella* Fast Detection result is considered presumptive positive and it is recommended to confirm it 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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2. International Organization for Standardization. 2004. ISO 11731-2:2004. Water quality - Detection and enumeration of *Legionella* -- Part 2: Direct membrane filtration method for waters with low bacterial counts. International Organization for Standardization, Geneva, Switzerland.

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7. Pilar Delgado-Viscogliosi et al. 2005. Rapid Method for Enumeration of viable Legionella pneumophila and Other *Legionella spp* in Water. Applied and Environmental Microbiology, Vol. 71, No 7, p.4086-4096.

Notice to purchaser: Use this product only for environmental testing

Code: **Lot number:** **Expiry date:**	For **technical assistance** please contact: Biótica, Bioquímica Analítica, S.L. Science and Technology Park, Jaume I University Building Espaitec 2, ground floor, lab 2 E12071 – Castellón, Spain www.biotica.es info@biotica.es Tel.: +34 964108131 Fax: +34 964737790	biótica® FAST DETECTION FOR LIFE