



Legipid[®] Legionella Fast Detection

Reference:

311-10-04

Leaflet

Test for rapid detection of *Legionella spp* in water samples, based on the combination of immunomagnetic capture and enzyme immunoassay (CEIA).

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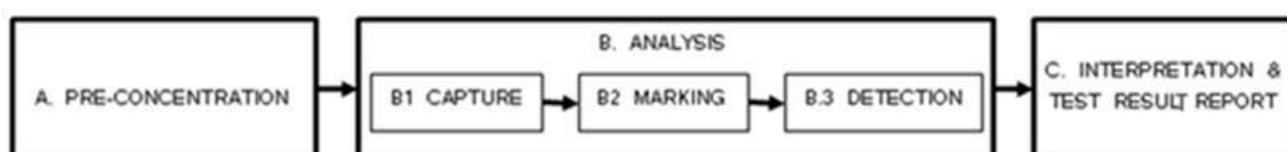
X. REFERENCES

I. INTRODUCTION

Legipid® Legionella Fast Detection (Cat. No. 311-10) It is a simple and quick test for presumptive detection of *Legionella* spp in tap, natural and industrial water. The test combines magnetic immunocapture and enzyme-linked immunoassay (CEIA) with enzymatic colorimetric reaction for quick 1 hour test, after pre-concentrate a sample.

II. TECHNOLOGY BEHIND THE TEST Legipid® Legionella Fast Detection

The original sample of water is concentrated by filtration or the like, and this prepared sample is eluted and dispensed into a cuvette to be analyzed by the method CEIA. A suspension of magnetic particles that bind to *Legionella* is added. If *Legionella* cells are present in the prepared sample, they will bind to the antibodies immobilized on magnetic particles to form complex bacteria / particle. As these complexes may be separated by a magnet, they are easily washed and resuspended. The complexes are incubated with an anti-*Legionella* antibody conjugated with an enzyme, to form labeled complexes. After washing the *Legionella* / particle complexes are visualized colorimetrically, when the enzyme substrates are added. This test includes the following 3 main stages:



III. KIT REAGENTS AND COMPONENTS

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

Reagent/Component	ID	Amount
Eluent	L0	1 flask (1050 mL)
Capture reagent (immunomagnetic particles)	L1	1 flask (105 mL)
Wash buffer	L2	2 flask (2 X 940 mL)
Enzyme conjugated Anti-Legionella antibody	L3	1 flask (105 mL)
Enzymatic cosubstrates	L4	35 tetra dose (35 x 5 ml)
Stop reagent	L5	1 flask (11 mL)
Cuvette	CB	100 units

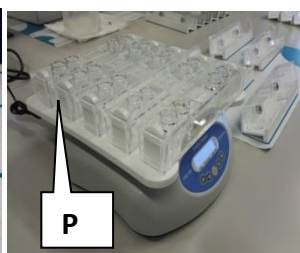
The concentrator **MP4-Hunter (311-MP4-00)** contains the elements listed in the following table:

MP4-Hunter (ref. 311-MP4-00), 1 unidad		
Component	ID	Amount
Magnetic holder for 4 cells	311-MP4-MH	5
Insertable holder for 4 cuvettes	311-MP4-CH	5
Clamping support	311-MP4-BB	5
Mat positioner (*)	311-MP4-TC	1
Platform(**)	311-MP4-P	1
Orbital Shaker (**)	311-MP4-AGT	1

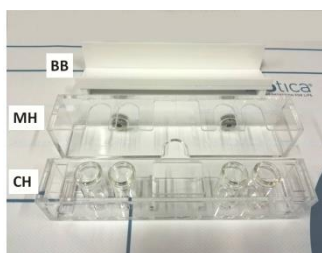
(*)Avoid excessive proximity between magnets. If not available, maintain a distance of at least 12 cm between hubs. (**) Not supplied if you opt for the manual stirring during the test.



311-10 CB



311-MP4-00



Components MH, CH and BB can be separately acquired as a set under reference MP4-SP.

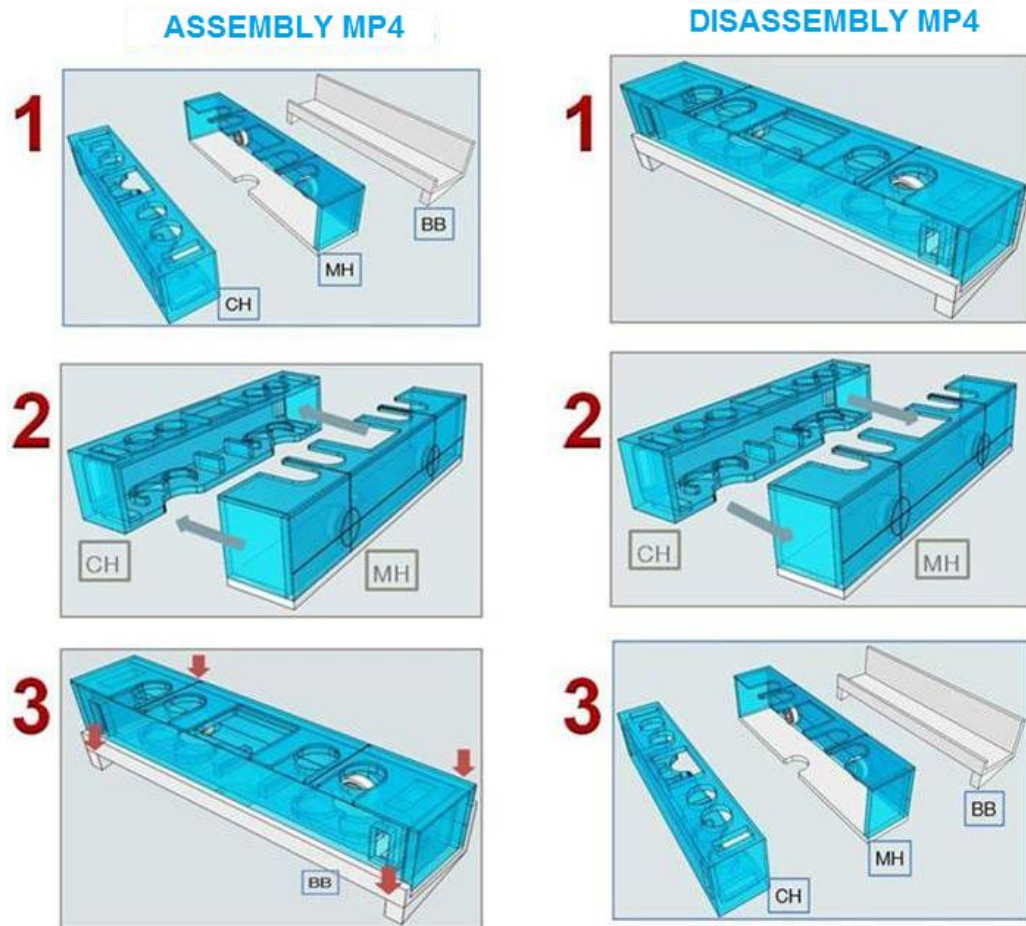
The assembly and disassembly of the concentrator is as follows:

ASSEMBLY MP4

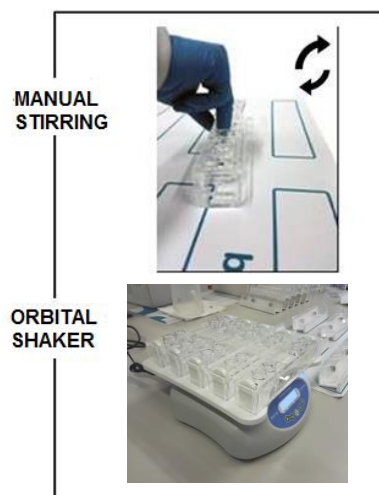
Insert cuvette into the holder (CH). Enter each cuvette holder (CH) in a magnetic holder (MH). Fit the unit in its clamping support (BB) (pressure slightly ahead and push it down).

DISASSEMBLY MP4

Disengage the clamping support (BB). Separate the cuvette holder (CH) of the magnetic holder (MH).



The holder with inserted cuvettes (CH) can be either manually shaken on the positioning mat (311-MP4-TC) or using an orbital shaker (311-MP4-AGT).



IV. EXPIRY AND STORAGE

Once received, the kit is stored between +2°C and +8°C, preferably at +4°C. The expiration of the reagents, properly stored, is **5 months from the date of manufacture**. All reagents include their own batch number and storage conditions. These conditions are also found in the packaging. In addition, the protocol includes code, lot number and expiration date, ensuring the traceability of all reagents. You can ask the manufacturer for a certificate of analysis.

V. MATERIAL NEEDED BUT NOT PROVIDED

- ◆ Graduated bottle with screw cap, for elution of the filter.
- ◆ Glass fiber filter, 2.7µm pore diameter, for use as a pre-filter (*).
- ◆ Membrane filter, 0.4µm pore diameter in the case of polycarbonate filters (**).
- ◆ Container for residue.
- ◆ 10-100µl pipettes, 100-1000µl and 1-5ml.
- ◆ Filtration apparatus (***), to pre-concentrate water samples by membrane filtration.
- ◆ Optional: Semi micro cuvettes for spectrophotometer reading (ref.511-10-04, box 100 units).
- ◆ Optional: Primelab (ref 911-10-PL).
- ◆ Optional: vortex mixer or sonicator to elute the filter (the elution can be done manually).

(*) *The use of a glass fiber filter of 2.7 micron pore size as prefilter for water samples is recommended only for filtering very dirty samples.*

(**) *Smaller pore diameter is possible but it can hamper the filtration*

(***) *Note: Contact us for detailed information on equipment recommended by our technical department*

VI. PRECAUTIONS AND RECOMMENDATIONS

- ◆ This test should be performed by properly trained personnel.
- ◆ This test is designed for the following matrices: tap, natural and industrial water.
- ◆ The product is safe under normal conditions of use. Avoid contact with the eyes. If splashing might occur, wear safety glasses.
- ◆ Avoid skin contact by wearing gloves. (See MSDS)
- ◆ Attention: Certain isolates cannot be detected below 10⁶ CFU.
- ◆ Stable products. It is unlikely to react dangerously under normal conditions of use.
- ◆ The product should be disposed of in accordance with current regulations. Dispose of empty containers through the process of recycling or waste disposal.
- ◆ The assay performance depends on strict compliance with the following practices, especially regarding to the correct execution of the protocol:
 - Do not use reagents after the expiration date.
 - Using as negative control the same eluent (L0) used in sample preparation (elution).
 - Use a negative control (L0 reagent) for each batch of test.
 - Bring test reagents (18-26 ° C) at least 30 minutes before use.
 - Shake the reagent L1 before use, to the homogeneity of the magnetic particles.
 - Run carefully washing steps (reagent L2).
 - **The cuvettes are disposable. Do not reuse.**
- ◆ Leave to warm at least for 30 minutes the following amounts of reagents per test to be performed:
 - L0: 10 ml
 - L1: 1 ml per test (before taking the relevant ml shake the original reagent bottle L1 until a completely homogeneous suspension).
 - L2: 18 ml
 - L3: 1 ml
 - L4: each flask is for 4 tests.
 - L5: 0.1 ml
- ◆ **The tempered volumes must not be stored in a refrigerator again. Discard.**
- ◆ **Allow the required amount of L3 to reach room temperature protected from light.**
- ◆ Use the positioning mat (311-MP4-TC). If not, keep a distance among concentrators of at least 12 cm.
- ◆ For highly colored samples: after emptying supernatants, the assembled MP4 module can be tilted forward up to 45 ° to allow the residual liquid to drain to the bottom of the cuvettes, in the opposite side of the retained magnetic beads. This allows the elimination of this liquid by using a pipette.
- ◆ **The reagents are supplied in excess. Do not use of any reagent excess. Do not mix batches.**

VII. PROTOCOL

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

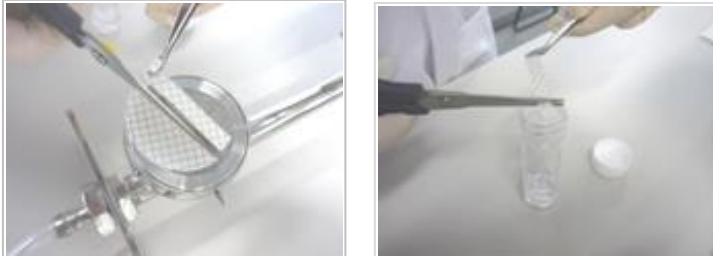
A. Sample preparation

1. Collect the sample volume of the original water to be concentrated (e.g. by filtration).
2. Add 10 ml of eluent in a flask. Use L0 as eluent.
3. Filter the collected volume using a membrane filter (*). For very dirty samples you can use a glass fiber pre-filter of 2.7 µm pore diameter placed on top of the filter.

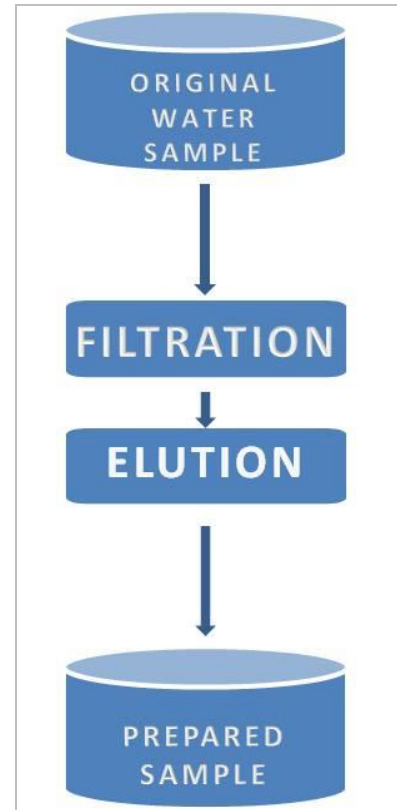
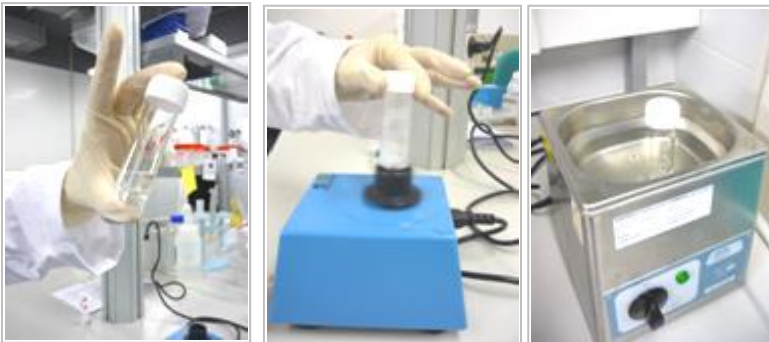
(*) AOAC PTM validation used 0.4 µm



4. Carefully separate the filter from the filtration system and put it into the flask with the eluent prepared in step 2. Optionally filter can be cut into pieces with scissors. If you have also used the pre filter, please remove it and discard.



5. Elute the filter by shaking. This agitation can be:
 - a. Manual (2 minutes)
 - b. Vortex (2 minutes)
 - c. Ultrasonic bath (5 minutes)



Note:

For each sample batch, a negative control is performed with the same reagent used eluent (L0)

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

The eluted sample is called prepared sample.

For an optimal recovery, shake this sample just before transferring to the cuvette.

B. Analysis with the kit Legipid® Legionella Fast Detection

Before starting the test:

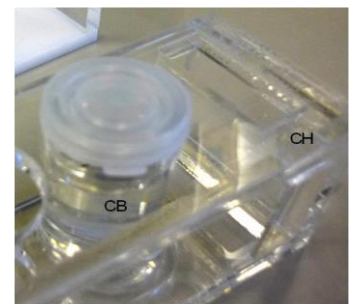
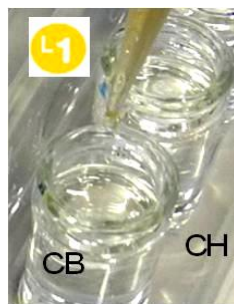
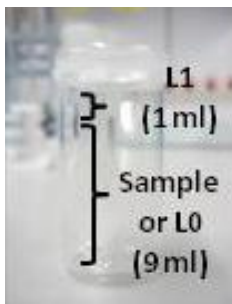
- **Prepare only the amounts of reagents needed and let 30 minutes at room temperature before use.**
- Insert the cuvettes (CB) in its support (CH), as many as trials are going to be performed and another one for the control.

If there is no shaker (AGT) with platform (P): manually manipulate the CH on the mat (TC).

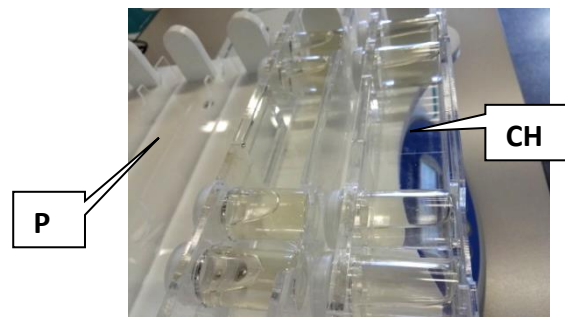
B.1) CAPTURE STAGE

1. Add each **prepared sample (9 ml)** in the corresponding cuvette (T). Then add L0 (**9 ml**) to the control cuvette (C) (just one control per testing batch). Apply to all cuvettes all the steps that follow.

2. Gently shake the L1 by repeated inversions until a completely homogeneous suspension. Resuspend by gentle and repeated pipetting, and then add **1 ml** in each cuvette. **PLACE THE PLUGS** into cuvettes.



3. Insert cuvette holders (CH) on the platform (P), if you have it, so that the **cuvettes are horizontal**. Stir at **80 rpm for 15 minutes** on the orbital shaker (**manual: on the mat in gentle sway shake 3 times every 3 minutes**).

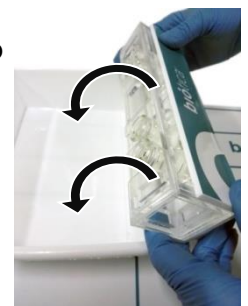


4. Remove the cuvette holders (CH) from the platform (P), if you have one, and **REMOVE AND DISPOSE THE PLUGS** (place the cuvettes upright). Assemble MP4 (see p. 4) and place in position on the mat (TC). Wait 5 minutes to retain the magnetic particles.

5. Discard supernatant by emptying **the cuvette on the opposite side to magnets**, by tipping this assembly up.

6. Disassemble the MP4 and insert the cuvette holder (CH) upright on the platform (P) on the orbital shaker (**manual: on the mat**). Then add **4.5ml of reagent L2 in each cuvette**.

7. Shake **WITHOUT PLUGS** at **350 rpm for 10 seconds** (**manual: on the mat in vigorous shake swing**) to resuspend the particles.



8. Remove the cuvette holders (CH) from the platform (P) - if you have it -, assemble the MP4 and place it in their position on the mat (TC) and wait **3 minutes** to retain the magnetic particles.

9. Discard supernatant emptying the cuvettes on the **opposite side to the magnets**, by tipping the assembly up.

B.2) MARKING STAGE

1. Disassemble the MP4 and insert the cuvette holders (CH) upright onto the platform (P) in the orbital shaker (**manual: on the mat**). Add **1 ml** of reagent L3 in each cuvette.

2. Shake **WITHOUT PLUGS** at **250 rpm** for **10 seconds** (**manual: on the mat in vigorous shake swing**) and then at **80 rpm** for **10 minutes** (**manual: gentle sway every 2 minutes for 10 minutes**).



3. Separate cuvette holders (CH) from the platform (P), if you have one, and assemble the MP4. Place them in their position on the mat (TC) and wait **3 minutes** to retain the magnetic particles.

4. Discard supernatant by pouring on the **opposite side to the magnets**, by tipping up the assembly.

5. Disassemble the MP4 and insert the cuvette holders (CH) upright onto the platform (P) in the orbital shaker (**manual: on the mat**). Then **add 4.5ml of reagent L2 in each cuvette**.

6. Shake at **350 rpm** for **10 seconds** **WITHOUT PLUGS** (**manual: on the mat shake in energetic swing**) to resuspend the particles.

7. Separate cuvette holders (CH) from the platform (P), if you have it. Assemble the MP4 and place them in their position on the mat (TC). Wait **3 minutes** to retain the magnetic particles.

8. **Repeat steps 4, 5, 6 and 7 (of this section B.2 MARKING STAGE) twice**

B.3) DETECTION STAGE

1. Discard supernatant by pouring on **the opposite side to the magnets**, by tipping up the assembly. Disassemble the MP4 and insert the cuvette holders (CH) upright onto the platform (P) in the orbital shaker (**manual: on the mat**).

2. Prepare the reagent L4 (one vial for each 4 trials): unseal the protective cap and remove it. Press the piston to be fully inserted into the shutter. **Shake the mixture vigorously for 10 seconds. Once the reagent L4 is prepared, it should be used immediately.**



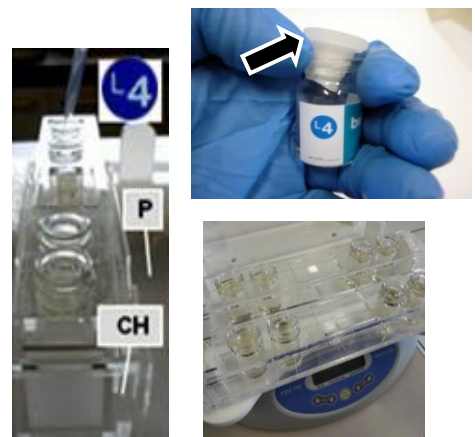
3. Open the vial of L4 just before use. To do this remove the shutter with the piston inserted, applying light pressure with the thumb side.

4. **Immediately add 1 ml pipette to each cuvette**. Shake **WITHOUT PLUGS** at **80 rpm** for **2 minutes** (**manual: on the mat in gentle sway**). Start shaking at **250 rpm** for the first **10 seconds** (**manual: swing energetically**) to resuspend the particles.

(Note: count time from the first addition of L4)

5. Stop shaking. Add **100 µl of L5** to each and every one of the cuvettes and shake for **5 seconds at 80 rpm** (**manual: on the mat in gentle sway**).

(Note: add reagent L5 in the same order as the L4 was added)

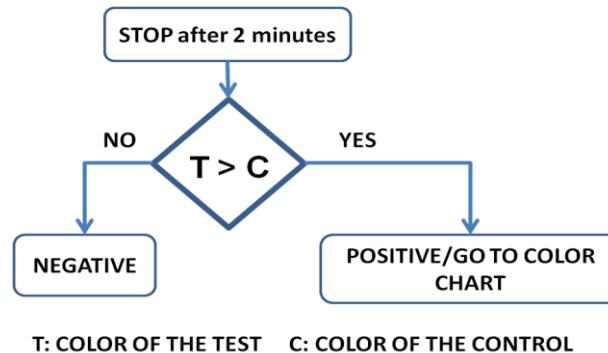


6. Separate cuvette holders (CH) from the platform (P), if you have it, and reassemble the MP4. Wait **5 minutes** to retain the magnetic particles.

C. RESULTS AND INTERPRETATION OF THE TEST

C.1. Visual Interpretation

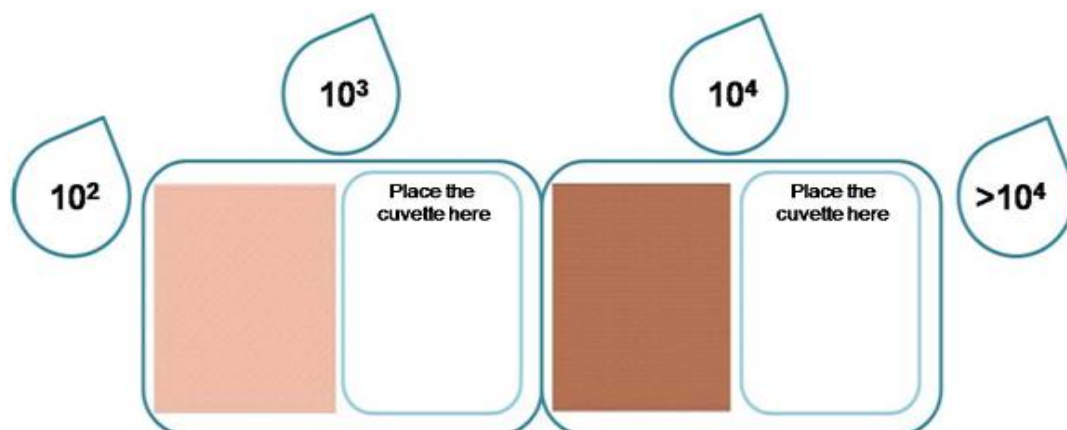
The visual interpretation of the test results is summarized in the following diagram:



The result of the test (T) is considered **POSITIVE** if the test (T) presents more color than the control (C) after **2 minutes** from the start of the colorimetric reaction. The overall estimate of the level of *Legionella* spp can be obtained by comparing the color of the test (T) **with the color chart**.

Color Chart Position the cuvette test (T) with the following color chart.

Results as CFU equivalent / volume examined



Below the first color of the card: up to two orders of magnitude (10^2 CFU_{eq} / volume examined). Below the second color of the card: up to three orders of magnitude (10^3 CFU_{eq} / volume examined). Over the second chart color: equal to or greater than four orders of magnitude (10^4 CFU_{eq} / volume examined).

The result of the test (T) is considered **NEGATIVE** if the test (T) has no color difference with the control (C) after **2 minutes** since the beginning of the colorimetric reaction.

C.2. Optical reading

(1) Transfer 1ml of the supernatants Control (C) and Tests (T), each in its corresponding cell reading. *Important note: Pipette 1ml of the supernatant from the opposite side to the magnet, being careful not to drag the particles retained by the magnet*

(If you have a Primelab device, follow the instructions; If you are using another colorimeter, continue with the following steps)

(2) Measure absorbance at 429 nm in a cell filled with distilled water. Set the absorbance at zero.

(3) Measure absorbance at 429 nm of the supernatant of the control (C), as reference. Readjust the absorbance to zero.

(4) Measure the absorbance of the supernatant of each test (T). Immediately read: always within 10 minutes since the end of the colorimetric reaction.

Note: If the the length of the light path is not 1 cm, the corresponding correction is necessary. Follow the manufacturer's instructions of your optical reader.

Negative results - tests of samples with relative absorbance readings below the cutoff value ($A_r = 0.04$ units) are negative and are reported as Not Detected.

Positive results — tests of samples with relative absorbance readings above the cutoff value ($A_r = 0.04$ units) are positive and are reported as Detected.

(5) For positive results, obtain the \log_{10} of relative absorbance.

(6) Estimate the concentration of *Legionella* spp in the volume examined entering the value of the \log_{10} of the relative absorbance (A_r) in the following formula:

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

(7) The result is obtained by applying the inverse transformation of the logarithm:

$$\text{Contamination (CFU}_{\text{eq}} / \text{volume examined)} = 10^y$$

Note: If desired, you can request an Excel spreadsheet programmed for automatic calculation of concentration.

At the end of the trial empty cuvettes and dispose. Do not reuse the cuvettes or the remains of reagents.

VIII. CONFIRMATION OF POSITIVE RESULTS

In the AOAC-RI certification, a positive result for Legipid® *Legionella* Fast Detection was considered a presumptive positive and was confirmed with standardized culture methods (for example ISO 11731: 1998) on a volume of 0.1-0.5 ml of the sample prepared. In the case of results that are inconsistent between Legipid® *Legionella* Detection Fast and the confirmation method, you should take steps to ensure the validity of results. The positive deviation may be associated with poor recovery of target bacteria by culture (viable but not culturable cells -VBNC-) microbiota which inhibits growth of *Legionella*, etc.), or insufficient compliance in protocolized washings step in the labeling stage.

IX. CHARACTERISTICS AND VALIDATION OF TEST

Legipid® *Legionella* Fast Detection kit is a quick and simple test for the detection of *Legionella* spp in water samples. The relative level of detection is 93 $\text{CFU}_{\text{eq}} / \text{volume examined}$ (LOD50). With optical reading the limit of detection is 40 $\text{CFU}_{\text{eq}} / \text{volume examined}$ and the quantification limit is 60 $\text{CFU}_{\text{eq}} / \text{volume examined}$.



Legipid® *Legionella* Fast Detection is validated by the AOAC-Research Institute in the Performance Tested Method Program to potable, natural and industrial water. Certificate No. 111101

X. REFERENCES

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6. Garcia, M. T., Jones, S., Pelaz, C., Miller, R. D. & Abu Kwaik, Y. (2007). Acanthamoeba Polyphaga feasible resuscitates non-culturable Legionella pneumophila after disinfection. Environ. Microbiol. 9, 1267-1277.
7. Pilar Delgado-Viscogliosi et al. 2005. Rapid Method for Enumeration of Legionella pneumophila feasible and Other Legionella spp in Water. Applied and Environmental Microbiology, Vol. 71, No. 7, p.4086-4096.

User warning: Use this product only for environmental analysis

<p>Lot No.:</p> <p>Expiry date from manufacturing:</p>	<p>For Technical assistance contact: Biótica, Bioquímica Analítica, S.L. Parque Científico y Tecnológico, Universidad Jaume I Edif. Espaitec 2, ground floor, lab 2</p> <p>12071 – Castellón, Spain www.biotica.es info@biotica.es Tel.: +34 964108131 Fax: +34 964737790</p>	
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IMPORTANT:

- This guide is not a substitute for the protocol, read the protocol carefully before starting the test.
- Do not use reagents after the expiration date.
- Use a negative control (L0 reagent) for each batch of analysis.
- Allow aliquots of the reagents to be used (18-26 °C) to temper for at least 30 minutes before use.
- The cuvettes are disposable. Do not reuse.
- Use the positioner mat corresponding to the magnetic particle concentrator used.
- Reagents are supplied in excess. **Do not use any leftover reagent.**

Sample preparation

- Filtering of 1 L of sample through a 0.4-micron polycarbonate filter.
- Put the filter (preferably cut) in 10 ml of L0 for the subsequent elution.
- Elute by agitation (vortex or manual) for 2 min or in the ultrasonic bath for 5 min.
- Transfer 9 ml of the eluate immediately after elution into the analysis cuvette.

Capture

- Shake gentle the **L1** reagent to homogenize the magnetic particles.
 - Check you have 9 ml of the sample in a cuvette and 9 ml of **L0** (control) in another cuvette.
 - Add **1 ml** of the **L1** reagent to both sample and control cuvettes.
 - Insert the plugs and place cuvettes in a horizontal position in the agitator.
 - Shake at **80 rpm** for **15 min**.
 - Remove plugs, discard them. **Retain** for **5 min**.
 - Discard the supernatant on the opposite side to magnet (**with retained particles**).
-
- Add **4.5 ml** of **L2** to both the sample and control.
 - Shake at **350 rpm** for **10 seconds**.
 - Retain** for **3 min**.
 - Discard the supernatant on the opposite side to magnet (**with retained particles**).

Labelling

- Add **1 ml** of **L3** to both the sample and control.
- Shake at **250 rpm** for **10 seconds** and continue at **80 rpm** for **10 min**.
- Retain** for 3 min.
- Discard the supernatant on the opposite side to magnet (**with retained particles**).

Washing

- Add **4.5 ml** of **L2** to both the sample and control.
 - Shake at **350 rpm** for **10 seconds**.
 - Retain** for 3 min.
 - Discard the supernatant on the opposite side to magnet (**with retained particles**).
- Carefully make the washing stages**
EXECUTE THIS STEP A TOTAL OF 3 TIMES

Detection

- Add **1 ml** of **L4** to both the sample and control.
- Shake at **250 rpm** for **10 seconds** and continue at **80 rpm** until **2 min**.
- Stop the reaction with **100 microliters** of **L5**.
- Shake, and then **retain** for **5 min**.
- Pipette **1 ml** of each supernatant to measure with spectrophotometer or color chart.