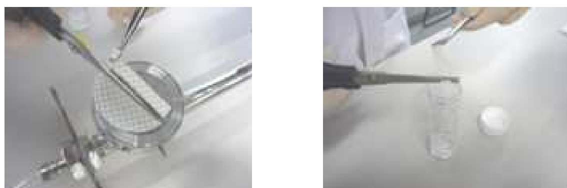
KIT BIOALARM LEGIONELLA

Biótica thanks you for your confidence in its product for rapid detection of Legionella. Below there is the protocol for the proper implementation of the Kit. If you have any questions or doubts, please contact us, we will be glad to assist you.

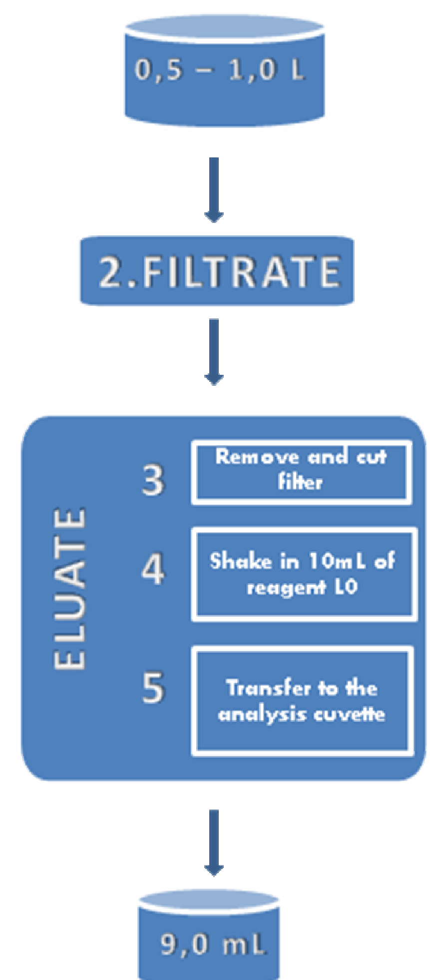
Sample filtration

1. Take 0,5 - 1,0 L of water sample from the selected point in the installation you wish to analyze.
2. Add 10ml of the L0 reagent in the graduated bottle that has been provided for this purpose.
3. Proceed to the collected volume filtration using a glass fiber pre-filter of 2,7 µm pore diameter placed on a nitrocellulose filter of 0,45 µm pore diameter or on a polycarbonate filter of 0,40 µm pore diameter.

4. Remove the glass fiber pre-filter and discard it. Then carefully remove the filter from the filtration system and cut it into several pieces with scissors. As it is being cut, deposit the pieces into the graduated flask with the L0 reagent previously prepared.



5. Elute the filter by shaking. Such agitation can be:
   a. Manual (2 minutes)
   b. Vortex (2 minutes)
   c. Ultrasound bath (10 minutes)



Recommendations:
In each batch of samples, an analysis of the L0 reagent (negative control) will be done.

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

Analysis using the Kit Bioalarm Legionella

Prepare materials

Let warm the reagents that are going to be used at room temperature for not less than 30 minutes.

If the cuvettes have been previously used, separate the magnets and add the L2 reagent up to the line 1. Cover and shake vigorously. Discard the contents.

A) CAPTURE

1. **Shake the L1 until a completely homogeneous suspension is got** and add it up to line 1.
2. Add the sample previously filtered and eluted up to line 3, being careful not let to fall the pieces of the filter into the cuvette.
3. Shake gently, with the lid on, once every 3 minutes, for 15 minutes.

4. Put the magnet as close as you can and wait 5 minutes to retain the immunomagnetic particles.
5. Empty the cuvette carefully, making sure you don't take the retained particles away.

6. Separate the magnet from the cuvette and add the reactive L2 up to line 2.
7. Shake gently WITHOUT lids until the particles are suspended again.

8. Put the magnet as close as you can and wait three minutes to retain the immunomagnetic particles.
9. Empty the cuvette, being careful not to take any retained particles away.

B) MARKING PROCESS

1. Remove the magnet, add all the content of one of the L3 vials and shake.
2. Shake gently WITHOUT lids every 3 minutes for 10 minutes.
3. Put the magnet as close as you can and wait 3 minutes to retain the immunomagnetic particles.

4. Discard the supernatant, being careful not to take any retained particles away.
5. Separate the magnet from the cuvette and add the L2 reagent up to line 2.

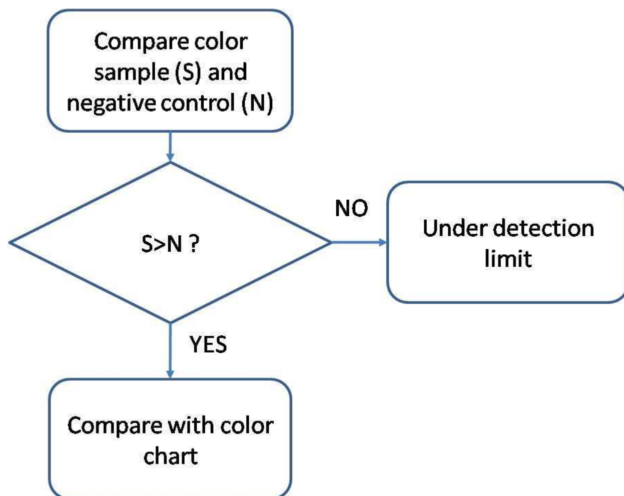
6. Shake gently WITHOUT lids until the particles are resuspended again.
7. Put the magnet as close as you can and wait 3 minutes to retain the immunomagnetic particles.

8. _Repeat steps 4, 5, 6 y 7 (of this section B)MARKING PROCESS) two more times._

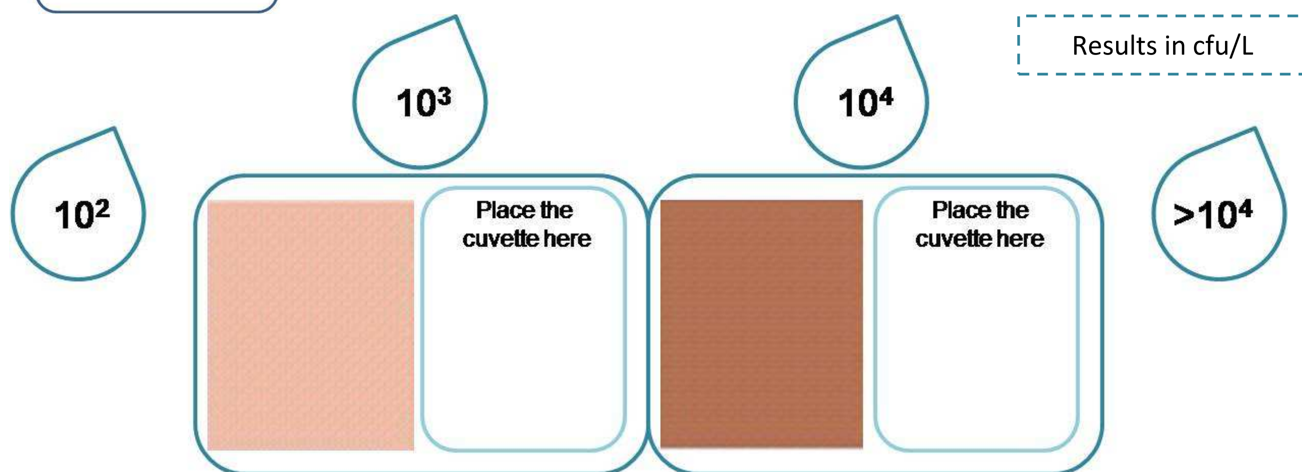
C) DETECTION

1. Add L5 reagent to a vial of L4 up to achieve the mark of 1.3ml (considering graduation of 0.1ml in the vial). Shake vigorously.
2. Empty the cuvette, paying attention not to drag the captured particles.
3. Separate the magnet from the cuvette and add all the contents of the dissolved mixture.

4. Shake gently WITHOUT lids, until the particles are resuspended. Wait 2 minutes shaking it gently meanwhile.
5. Add 3 drops of L6 and shake gently WITHOUT lids. Wait 1 minute.

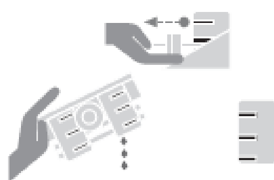


6. Bring the magnet up to the cuvette to capture the particles, wait for 5 minutes and observe the color obtained.
If the color of the sample is similar to the negative control, the concentration is below of the detection limit of the Kit. If the color of the sample is higher than the color of the negative control, compare it with the color chart.



Cleaning: If the user reuses the cuvette, we recommend cleaning it according to the following protocol.

1. Separate the magnet from the cuvette, resuspend and empty its contents.
2. Add the reagent L7 up to the line 1 and put on the lids.



3. Shake vigorously WITH the lids on.
4. Open the lids and discard the content. Dry the interior of the cuvette with absorbent paper. Before reusing, rinse the cuvette with L2 reagent according to this protocol.

NOTA: Do not use detergents

Code:
Lot number:
Caducity:

biotica
FAST DETECTION FOR LIFE

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