

Microbiological Challenge Testing

Microbiological tests were performed to challenge the New AquaGarde Formulation in a hot tub with Legionella.

Two separate tests were carried out for Legionella Pneumophila.

Analytical protocol test 1:

A first test was carried out with 4 samplings using a control formulation without active ingredients. A second test was carried out with the same protocol with 4 samplings, using the formulation containing the active ingredients.

Hot Tub Temperature : 35°C

Preparation:

Day +1 Clean hot tub and fill with fresh tap water. Heat water to correct temperature.
Day +1 addition of nutrients and inoculation of bacteria

Test:

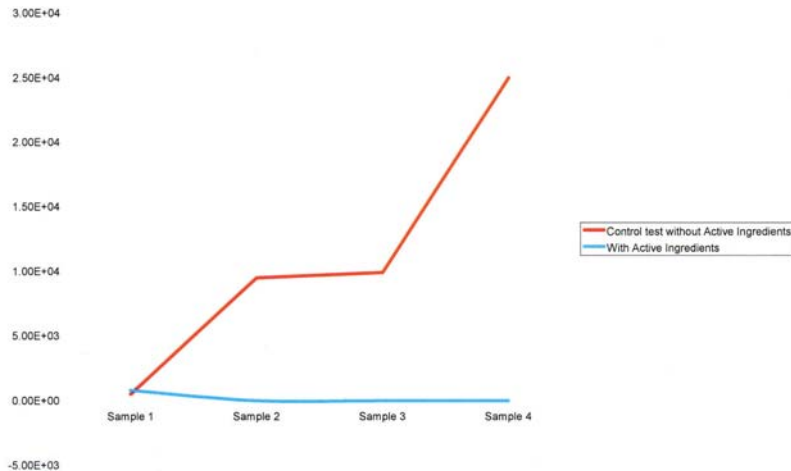
Day +2 **Sample 1** before addition of water treatment formulation
Day +2 Addition of water treatment formulation
Day +3 **Sample 2** after 24h incubation
Day +4 **Sample 3** after 48h incubation
Day +5 **Sample 4** after 72h incubation

Results:

Test 1 :

Legionella pneumophila UFC/l	Control test without Active Ingredients	With Active Ingredients
Sample 1	5.0E+02	8.0E+02
Sample 2	9.5E+03	<500
Sample 3	9.9E+03	<500
Sample 4	2.5E+04	<500

Graphic Representation of results of Test 1



Analytical protocol test 2:

A second test was carried out in order to establish growth at 37°C, the most prolific environment for bacterial growth. The aim of this test to simulate a longer period of testing, with more sampling and to reflect typical use of the formulation in a hot tub with regards to quantity and frequency.

Hot Tub temperature : 37°C

Preparation:

Day +1 Filling of hot tub and warming up to correct temperature.
Day +1 addition of nutrients and inoculation of bacteria

Test:

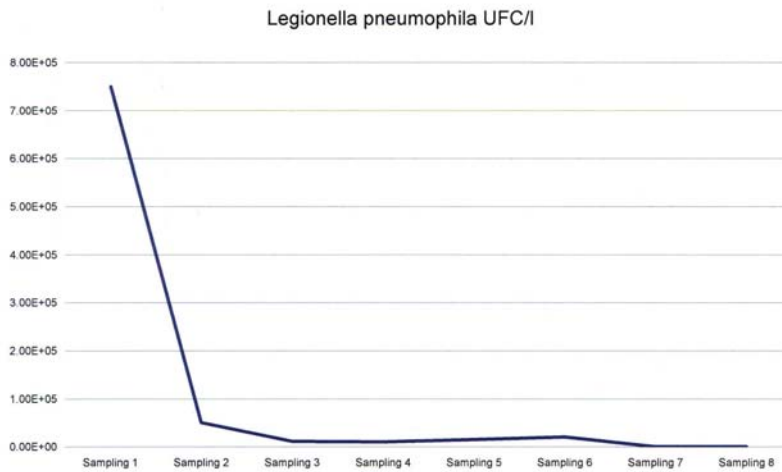
Day +2 **Sample 1** before addition of water treatment formulation
Day +2 addition of water treatment formulation
Day +3 **Sample 2** after 24h incubation
Day +4 **Sample 3** before addition of water treatment formulation
Day +4 addition of water treatment formulation
Day +5 **Sample 4** after 24h incubation
Day +8 **Sample 5** after 96h incubation
Day +9 **Sample 6** after 120h of incubation
Day +9 addition of water treatment formulation
Day +10 **Sample 7** after 24h incubation
Day +11 **Sample 8** after 48h incubation

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Results Test 2:

	Legionella pneumophila UFC/l
Sampling 1	7.5E+05
Sampling 2	5.0E+04
Sampling 3	1.1E+04
Sampling 4	1.0E+04
Sampling 5	1.5E+04
Sampling 6	2.0E+04
Sampling 7	<500
Sampling 8	<500

Graphic Representation of results:



Conclusion:

The findings show that the New AquaGarde Formula has a destructive effect on Legionella. It can therefore be concluded that when the Legionella bacterium comes into contact with the active ingredients of the Formula, these are destroyed and the formula successfully maintains the levels at virtually inexistent and immeasurable levels.

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