



KEMA TEST



## APPENDIX I EFFECTIVITY DETERMINATION OF THE PRODUCT 'AQUAFINESSE HOT TUB WATERTREATMENT'

### SPECIFICATION

The test-procedure applied is conducted in accordance with a test-protocol developed by TNO in the Netherlands. This protocol describes a hygiene-test in general. A modified version of this protocol is used in Germany to determine the potential of hygiene in whirlpools (LGA-Prüfrichtline IGS/2/1996). Application of this hygiene-test can determine if significant bacterial growth appears during use of that specific type of Whirlpool, especially during periods of non-use when significant bacterial growth can occur in stagnant zones available in tubes and piping.

This German Whirlpool test-protocol is adapted for hottub-systems because the water in these hottub-systems is being replaced, with fresh tapwater, less often compare to Whirlpool use. Chosen is for one period in which the dosed product should mitigate a bacterial concentration significantly. To determine the mitigation effectiveness of the product the hottub-system will be inoculated with the bacteria species *Pseudomonas putida*. ATCC 12 633 and some specific nutritious substances, as prescribe in LGA-Prüfrichtline IGS/2/1996. This bacteria species is representative for bacterial growth in bathing-systems in general. The bacteria species is obtained from the firm BioClear BV, NL-Groningen.

After inoculation and a 24 hours incubation period, the product 'AquaFinesse hot tub watertreatment' is dosed, and its mitigation effectiveness is determined after periods of 24 and 48 hour. In this hygiene-test the conducted circumstances are 'Worse-Case', this because normally the product 'AquaFinesse hot tub watertreatment' is dosed in accordance with an ozone installation or an UV-installation. From the chemical structure of the product 'AquaFinessehot tub watertreatment', it is not expected that the product will react with ozone. So, lower effectiveness as a result of this reaction with ozone is not expected.

The conducted test-procedure is as follow:

- 1 **(day 0)** take ozone installation out of operation and fill the hottub with tapwater. The operating water temperature should be adjusted to 35 degrees centigrade. The AquaFinesse has to be covert with plastic foil during the entire test-period
- 2 **(day 1)** turn on the water jets for 10 minutes, afterwards a 100 ml water sample (1) is taken in a sterile container for aerobic plate counts at KEMA

- 3 **(day 1)** enrich hottub water **(starting at a Monday 12:00)** with the following nutritious substances:
  - 0,005 ml Codfish-oil/L (substitute for skin grease)
  - 0,2 g Na-Stearate/L (soap)
  - 1 mg/L Peptone (substitute for dead skin cells)
  - $10^4$  cells of bacteria /ml (*Pseudomonas putida*. Code: ATCC 12 633) incubated for 12 hours at 30 degrees centigrade.
- 4 **(day 1)** turn on the water jets for a period of 10 minutes, afterwards a 100 ml water sample (2) is taken in a sterile container for aerobic plate counts at KEMA. Ozone installation should stay off-line. The circulation pump will be switched on for the next 24 hour
- 5 **(day 2)** after a period of 24 hour **(12:00)** a 100 ml water sample (3) is taken in a sterile container for aerobic plate counts at KEMA. Accordingly 500ml 'AquaFinesse hot tub watertreatment' per 1000 liter hottub-water is dosed, afterwards the water-jets are operated during 10 minutes (ozone installation stays off)
- 6 **(day 3)** after a period of 24 hours, water-jets are turned on for 10 minutes, afterwards a 100ml water sample (4) is taken in a sterile container for aerobic plate counts at KEMA
- 7 **(day 4)** after again a period of 24 hours, water-jets are turned on for 10 minutes, afterwards a 100ml water sample (5) is taken in a sterile container for aerobic plate counts at KEMA. Mitigation activity has to be optimal at this time. Ozone installation can be switched on
- 8 **(day 5)** after again a period of 24 hours, water-jets are turned on for 10 minutes, afterwards a 100ml water sample (6) is taken in a sterile container for aerobic plate counts at KEMA.

In the aerobic plate count nutrient agar was conducted in order to estimate the density of bacteria in the water samples. A total amount of five samples were plated at a nutrient agar petri dish and incubated at 30 degrees centigrade. After an incubation period of approximately 24 hours the viable microorganisms (seen as colonies) present were evaluated. For each sampling the analyses were carried out in triple and with a non-diluted, 10, 100 & 1000 dilution rate. The results obtained in the aerobic plate counts are presented in Colonyforming Units (cfu/ml). The company WWR International BV, NL-Roden, delivered sterile Nutrient agar petri dishes.

## TEST CLASSIFICATION

The proceedings of the German hygiene test for Whirlpools show positive results, if the viable microorganisms present in the water after filling the tub are  $< 1 \times 10^4$  cfu/ml for three weeks when the test is carried out twice a week. This result shows that no microbial growth has taken place into the Whirlpool system and piping during the periods of stagnation between the tests. Besides the standard for classification if water is contaminated by microbes and should be treated used by the Dutch (ARBODIENST) is  $\geq 1 \times 10^4$  cfu/ml. The classification of the effectiveness of the product 'AquaFinesse hot tub watertreatment' is therefore conducted according these standards. If the product 'AquaFinesse hot tub watertreatment' reduces the viable microorganisms present in the hottub significantly, indeed lower than  $1 \times 10^4$  cfu/ml, it can be concluded that the product is effective in the mitigation of microbial growth in hottub-systems.

### **Caution:**

*Given results of this hygiene-test showing overall aerobic counts, no relation can be made with specific bacterial counts like Legionella counts.*

## TEST RESULTS

The effectiveness of the product 'AquaFinesse hot tub watertreatment' was tested during the period 19 till the 23<sup>rd</sup> of January on site of the supplier Special Water Europe BV. The test bacteria *Pseudomonas putida* was delivered at KEMA with a microbial density of  $5.4 \times 10^8$  cells/ml and incubated at 30 degrees centigrade for three hours at the day of hottub inoculation.

For aerobic plate count determination, 100 $\mu$ L of the water sample and diluted water samples were plated on a sterile nutrient agar petri dish and incubated at 30 degrees centigrade for one or two days. Results of viable microorganisms (seen as colonies) after incubation are given in Table 1 and photographed in Appendix II.

Table 1 Aerobic count of bacteria *Pseudomonas putida* in the different water samples

<b><i>date</i></b>	<b><i>water sample</i></b>	<b><i>aerobic count</i></b> <b><i>(CFU/ml)</i></b>
19 Jan. 2004	1	2.4 x10 <sup>4</sup>
	2	3.5 X10 <sup>4</sup>
20 Jan. 2004	3	2.0 x10 <sup>6</sup>
21 Jan. 2004	4	4.8 x10 <sup>4</sup>
22 Jan. 2004	5	3.7 x10 <sup>3</sup>
23 Jan.2004	6	1.9 x10 <sup>3</sup>

## CONCLUSION

The aerobic count of the water present in the hottub-system is by addition of the product 'AquaFinesse hot tub watertreatment' reduced from 2000x10<sup>3</sup> tot 3.7x10<sup>3</sup> cfu/ml. This indicates that the results are corresponding with the test-classification of < 1x10<sup>4</sup> cfu/ml.

**From the preformed hygiene-test it can be concluded that the product 'AquaFinesse hot tub watertreatment' is effective for the mitigation and control of microbes in hottub-systems.**

The product is composed of non **biocidal** chemicals (mainly salts) and is safe for humans to be used in hot tubs at the prescribed concentrations which are clearly explained in the supplemented manuals.

## APPENDIX II OVERVIEW INCUBATION GROWTH PLATES

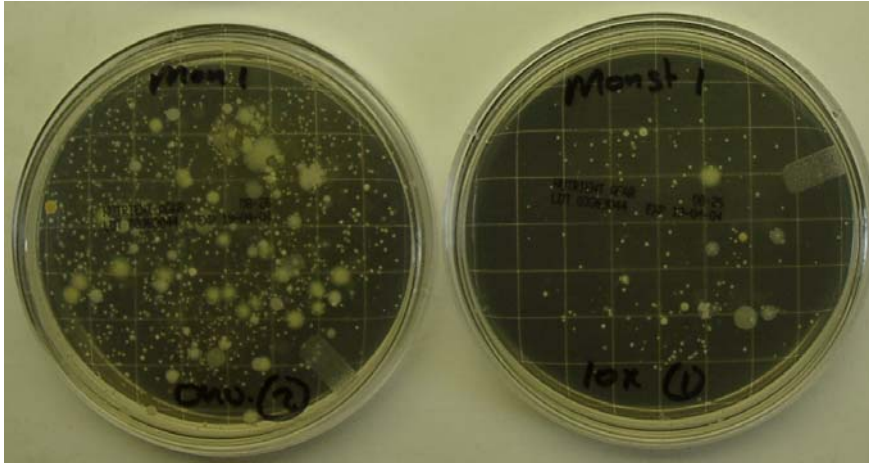


Figure 1 Water sample no. 1, dilution from left to right (non-diluted, 10x)

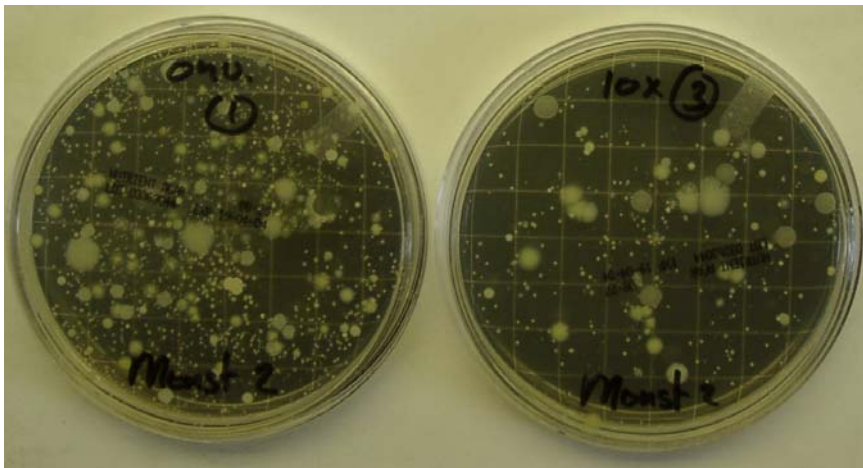


Figure 2 Water sample no. 2, dilution from left to right (non-diluted, 10x)



Figure 3 Water sample no. 3, dilution from left to right (non-diluted, 100x, 1000x)

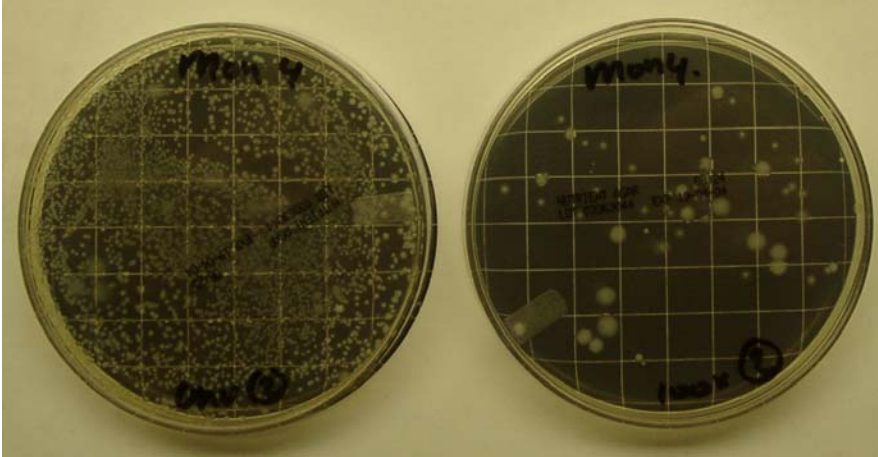


Figure 4 Water sample no. 4, dilution from left to right (not-diluted, 100x)

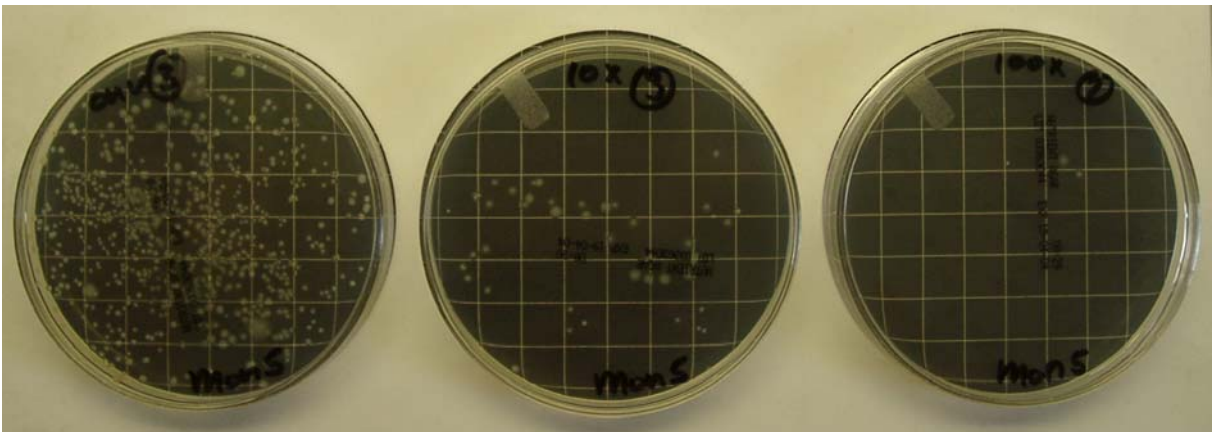


Figure 5 Water sample no. 5, dilution from left to right (not-diluted, 10x, 100x)

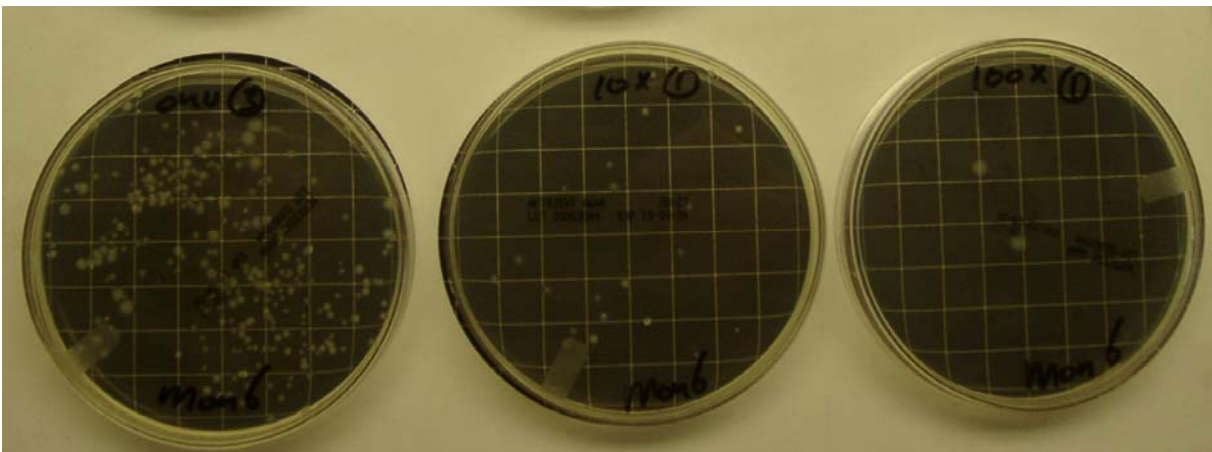


Figure 6 Water sample no. 6, dilution from left to right (not-diluted, 10x, 100x)