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To Whom it may concern

CQM device for Legionellae inactivation in water systems (Hospitals, Elderly houses, Hotels, etc.)

General

Legionella bacterium is an opportunistic environmental pathogen frequently isolated from hot drinking water. The main clinical manifestations of *Legionella* infection are acute pneumonia (called Legionnaires' disease) and Pontiac fever (a milder non-pneumonic flue like syndrome). The bacterium is mainly isolated from domestic hot-water systems (Atlas et al., 1995), cooling tower (Wery *et al.,* 2008), fountains (Hlady *et al.,* 1993), and similar disseminators that tap into public water supply. Other natural sources of *Legionella* include freshwater ponds, creeks, oxidation ponds, etc. (Shelton *et al.,* 2000). The first report on *Legionella* was published by Tatlock in 1944 (Tatlock 1944), later rediscovered by McDade during the outbreak among people attending a convention of the American Legion in Philadelphia (July, 1976) (McDade *et al.* 1977). Since then an extensive number of publications emerged on its distribution, identification, serogrouping, ecology, epidemiology, molecular biology and clinical aspects (Fliermans *et al.,* 1981; Martinelli *et al.,* 2000). Currently there are at least 50 *Legionella* species and approx. 70 serogroups known to us (Grattard *et al.*, 2006). Examination of the international published literature on *Legionella pneumophila* serogroups prevalence around the globe reveals that widely held isolated tap water serogroup is *Legionella pneumophila* serogroup 1 (Helbig *et al.,* 2002; Tateyama *et al.,* 2002; Harrison *et al.,* 2007; Mika *et al.,* 2005; Yu *et al.,* 2008). Specific reports from countries around the Mediterranean Sea: Turkey, Greece, Croatia, Italy, France and Spain also point on *Legionella pneumophila* serogroup 1 as the drinking water most prevalent serogroup isolate (Polat *et al*., 2007; Alexiou-Daniel *et al*., 1996; Kuzman 1996; Boccia *et al*., 2005; Chiarini *et al*., 2008; Campese *et al*., 2007; Rivera *et al*., 2007).

In Israel, Haifa Public Health laboratory (the only accredited and certified laboratory by Ministry of Health on *Legionella*) surveys on *Legionella* spp. prevalence in drinking water continuously. The most prevalent

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Legionella pneumophila serogroup isolated in Israel is serogroup 3 (Yarom *et al*. 2010). Several cases of Legionellosis were detected in hospitals, but no real epidemics occurred in Israel. Due to the nature of hospitals, elderly homes, and other public sites that encounter large numbers of people, continuous monitoring as well continuous disinfection is required. The most economic mean of efficient disinfection against *Legionella* spp. is chlorination as this bacterium is sensitive to chlorine (Muraca *et al.* 1987) and other oxidants. Among other means for *Legionella* bacterium eradication is temperature increase to 60-70°C and drainage of the water for a certain time interval. Figure 1 represents *Legionella* behaviour at different temperatures.

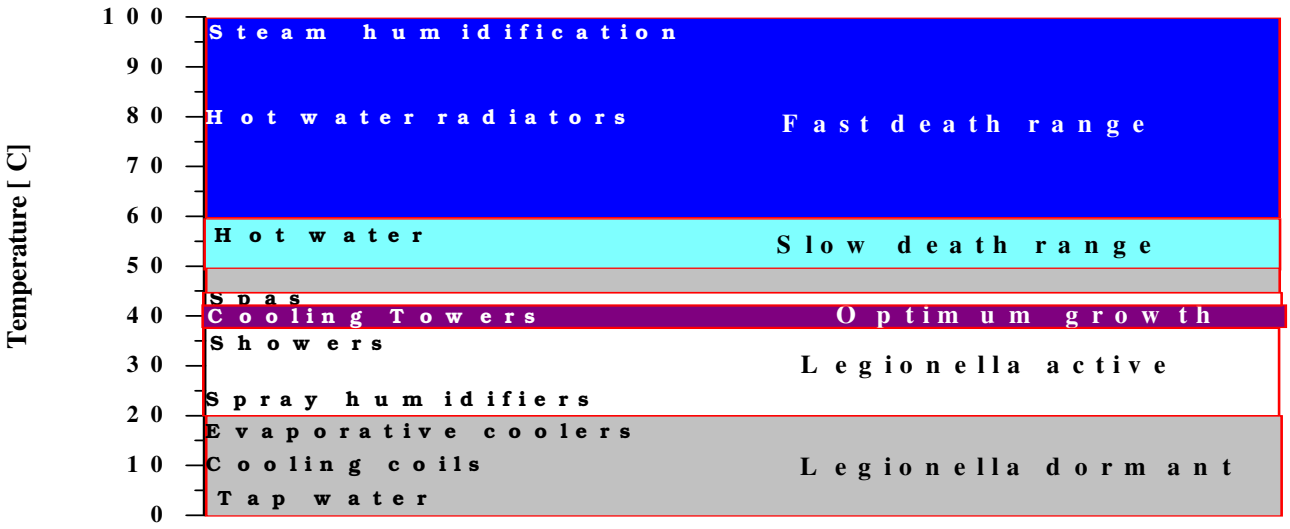

Figure 1. *Legionella* bacterium survival vs. water temperature.

The CQM device

CQM developed a device intended to inactivate *Legionella* bacterium, found and colonizing hot water systems, through a process called electrolysis. Under the device specifications, it produces free chlorine and possible other oxidants (ozone, etc.) that inactivate/kill bacteria in water at 40°C. There were several questions that experimentally had to be answered:

1) Is the device efficient in annihilating *Legionella pneumophila* in water at 40°C or less?
2) Does the device produce bromates at high level of bromides?

To answer these questions, 2 experiments were carried out through seeding of *L. pneumophila* at high concentration (10^7-10^8 CFU/ml) into the CQM device and followed for its potential inactivation as a function of time. *L. pneumophila* enumeration was performed at the different time intervals by Haifa Public Health

Laboratory (Dr. Rachel Yarom, Head). The device was also challenged with bromine at 0.1 mg/l to test bromate formation (Prof. Lahav, Technion).

Experimental Steps

1. Device cleaning and disinfection by continuous recirculation with 4-5 ppm free chlorine (produced by the device itself) at 90oC for one and a half hour.
2. Removal of the volume and refilling with laboratory tap water.
3. Operation of the system for 90 minutes with micronic filter for turbidity removal.
4. Turbidity test of regular tap water.
5. Turbidity test of device filtered water.
6. Enumeration of *L. pneumophila* seed volume before addition to device.
7. Introduction of *L. pneumophila* seed volume to performing device (only while pump is active) at 35^{o}C to achieve good dispersion of the bacteria.
8. After 60 minutes, sampling for L. pneumophila and enumeration.
9. Activation of the electrolytic system to achieve a chlorine level of 0.5 ppm.
10. After 60 minutes, sampling for *L. pneumophila* presence.
11. Sampling at 60 minutes intervals for L. pneumophila, residual chlorine and turbidity for the next 6-7 hours.
12. Next day (after 24 hours) the tests were performed for additional 6-7 hours.
13. Experiment termination.

Results

Three experiments were done – each one for 2 days (with sampling every hour for 8 hours).

In each experiment the device was challenged with 4 x 10^5 CFU/liter of *L. pneumophila* from fresh overnight culture (~ 10^7 CFU/ml).

The background flora was < 1 CFU/liter. Background flora is defined as different bacteria capable to grow on *Legionella* medium (BCYEα + cysteine + antibiotics).

In the 1st experiment seeded *Legionella pneumophila* was reduced to "zero" immediately (~ 30 minutes) since device activation. During the 2 days of experiment no *Legionella* & no background microorganisms were detected.

In the 2nd experiment, again, the *Legionella pneumophila* was reduced to "zero" immediately. However background microorganisms were detected in the first two hours but dropped to < 1 CFU/ml for the remaining time interval. bacteria was found and disappeared after few hours.

On the 2nd day *Legionella* and background bacteria were completely eliminated.

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In the 3rd experiment sodium thiosulfate was added in order to neutralize free chlorine (to prevent Legionella inactivation inside the device prior to experimental run. Therefore the system produced chlorine levels of 0.2-0.3 only unable to increase this concentration. In spite of, seeding *L. pneumophila* at 4 x 10^5 CFU/liter after 60 minutes Legionellae count was reduced to < 1 CFU/liter and continued to stay at this level for the next day when the experiment was terminated (Figure 2). However, background bacteria increased significantly from 1.6 x 10^4 CFU/liter in the second hour to 3 x 10^4 CFU/liter in the next day and remained stable. The dominant background bacteria were identified as *Pseudomonas aeruginosa* by Bactek.

The re-growth of background flora is important as some of these bacteria are opportunistic pathogens and certainly not acceptable in hospital waters or other institutions. Therefore other 2 experiments were carried out to test for background flora inactivation by the CQM device.

We decided to conduct another experiment to test the effectiveness of the treatment CQM device.

In the 4st experiment we have initiated a system restart when the amount of background bacterial flora is 3 x 10^4 CFU/liter. Chlorine level was adjusted to 0.5 ppm (as free chlorine). In the first hour the background bacteria was eliminated completely <1 CFU/ml.

In addition the device was activated continuously for two weeks. Background bacteria were tested once a day. No background bacteria were detected (< 1CFU/ml).

In the 5st experiment CQM device was stopped and left to stay for one week to see if any background bacteria may develop during this time interval. After one week of inactivity no background bacteria were found (<1 CFU/ml).

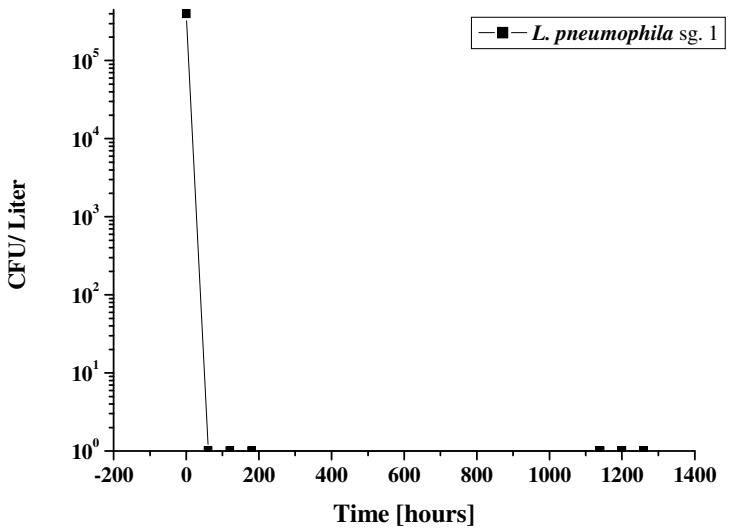

Figure 2. *Legionella pneumophila* sg 1 reduction by CQM device at 35°C after two days of continuous operation.

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Bromate

The system was challenged with bromide (Table 1) in order to test bromate formation under various conditions (CQM device). The results showed that relatively low bromate concentrations were formed after device activation (7.6 and 11.4 μg/L).

$$Br^- + O_3 \rightarrow BrO_3^-$$

Table 1. Bromate formation under various operational conditions

Sample No.	Experimental Conditions	Bromide (μg/L)	Bromate (μg/L)
1	No bromide added	72	0
2	Bromide +*Legionella*	102	0
3	Bromide + *Legionella*	104	0
4	After device activation	119	7.6
5	After device activation	123	11.4

It should be emphasized that these results are very preliminary and were performed under the experimental conditions throughout the *Legionella* test performed at Technion.

EPA data on bromate level guidelines is listed bellow for comparison with our preliminary results.

Contaminant	MCLG[1] (μg/L)	MCL[2] (μg/L)	Potential Health Effects from Ingestion of Water	Sources of Contaminant in Drinking Water
Bromate	zero	100	Increased risk of cancer	Byproduct of drinking water disinfection

[1]Maximum Contaminant Level Goal (MCLG)

[2]Maximum Contaminant Level (MCL)

Conclusions: After two weeks of activating, no appearance of background bacteria after the device stops its Activity for 7 days.

1. CQM device inactivates *Legionella* very fast (<1 CFU/liter in less than 1 hour) therefore providing free Legionellae water.
2. CQM also inactivate background microorganisms supplying additional disinfection efficiency.
3. CQM device has to be continuously operated in order to prevent bacterial regrowth due to organics oxidation. If the device is inactive then it should be disconnected from the main water supply line by

physical means (taps, valves, etc.). These measures should be taken in consideration as related to some cases of background microflora development.

Summary and Recommendations

Our laboratory of "Environmental microbiology" at Technion tested the CQM device for its ability to inactivate the human pathogen *Legionella pneumophila* sg.1 and other bacteria in tap water (at 35^{o}C).

The results show fast inactivation of *Legionella* and other bacteria by CQM device with continuous absence of the bacterium up to 1400 hours (when the experiment was terminated).

Additional tests performed in Austria (Institute fur Hygiene, Gratz, Prof. Dr. E. Marth) at water temperatures of 41.1 to 53^{o}C showed that no *Legionella* were detected in 100 ml. The device was installed after findings of 4600 CFU/100 ml of the Austrian Ministry of Health in December 2007.

The CQM device was mounted 0n March 2009 at Bnai-Zion hospital with continuous operation at water temperature of 40oC. It was installed after finding *Legionella* at the hot water in values up to 2,000 CFU/liter, on 26th of February 2009.

According to the performed tests during one year, by the Public Health Laboratory, Haifa - no *Legionella* was found in the treated water.

As a conclusion from all the experiments, we can state that the CQM device is effective against *Legionella* bacterium and background bacteria in tap water at different temperatures (35-50^{o}C) due to its continuous production of residual chlorine.

It is recommended to keep level of 0.5 ppm of the Chlorine to ensure destruction of background bacteria.

To prevent development of Legionella and background microorganism development, in case the device failure, it is recommended to increase, temporary, the temperature of the water due to the Ministry of Health demands.

The device should be disinfected by self production of chlorine or by streaming hot water before reactivation.

Also it is recommended to install monitoring system, remote control which alerts when there is decrease in the amount of residual chloride in the water.

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