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Exposure assessment for bacterial load and risk assessment for Legionella in water systems using Bactiquant®-water

SUMMARY:
Bactiquant®water provides a rapid comparative and precise report for bacterial loads in water systems. The technology can be used to identify hotspots in water systems with an increased risk of adverse health effects associated with high bacterial loads and *Legionella* infestations. Statistical analysis of data from investigations of Critical Care Facilities has demonstrated that the technology can be used as a rapid Risk Assessment Tool for *Legionella* in hot water systems. A tentative cut of value of a BQW-value = 40, in non-chlorinated water, has been established for a good hygienic standard in a water system. This threshold level can be used to evaluate water treatment technologies that reduce bacterial presence and proliferation as well as verification of water hygiene in Professional Quality Systems ISO 22000/HACCP.

POOR WATER QUALITY AND HEALTH EFFECTS:
In general, hot water contaminated with bacteria can cause allergic reactions such as itching and blushing skin. It can also lead to smell, discoloration and development of biofilms reducing water heating efficiency and microbial induced corrosion. In water samples with high bacterial loads the risk of finding the bacterial toxin – endotoxin increases (Evans et al., 1978; Martin and Daley., 2001) Endotoxin is a heat stable toxin, which is present when bacterial cells are disrupted, and can cause adverse health effects if swallowed or inhaled as bio aerosols. In large quantities endotoxins can cause hemorrhagic shock and severe diarrhea, in smaller quantities endotoxins can cause fever. In addition to adverse health concerns associated with endotoxin, high bacterial loads in water systems associated with poor water quality, also increases the risk of *Legionella* (Bartram (Ed) et al., 2007) and other pathogenic bacteria such as *Pseudomonas,* and atypical mycobacteria.

THE LEGIONELLA BACTERIA:
Legionella is a common bacteria found in natural environments. The genus includes several species and serotypes that can cause legionellosis, an acute respiratory infection. *Legionella* can multiply to very high numbers in water systems. Operation of cooling towers and potable water systems can generate aerosols (liquid droplets in air) containing high levels of *Legionella,* causing legionellosis.

ECOLOGY OF LEGIONELLA:
Water alone is insufficient to allow *Legionella* to grow. In water alone they can survive but they cannot proliferate. The survival strategy for *Legionella* is to enter the biofilm where it can live protected. The presence of other microorganisms in the biofilm allows *Legionella* to grow. The biofilm facilitates nutrient and gaseous exchange, and protects *Legionella* not only from biocides but also from periodic increases in temperature and attempts at physical removal, especially in areas where surfaces are scaled or corroded (Bartram et al., 2007). Pieces of biofilm can spread and establish growth in the water system. Protozoa is an important vector for *Legionella* proliferation. Amoebae graze on biofilm and hereby uptake *Legionella*. Inside the amoebae, *Legionella* proliferate and eventually are released in high numbers into the water (Illustration 1) (Winiecka-Krusnell and Linder, 2007; Hoffman (Ed) et al., 2007). In general, water systems exhibiting poor water quality support a high level of bacterial presence by providing nutrients and biofilms increasing the risk of developing severe *Legionella* infestations.

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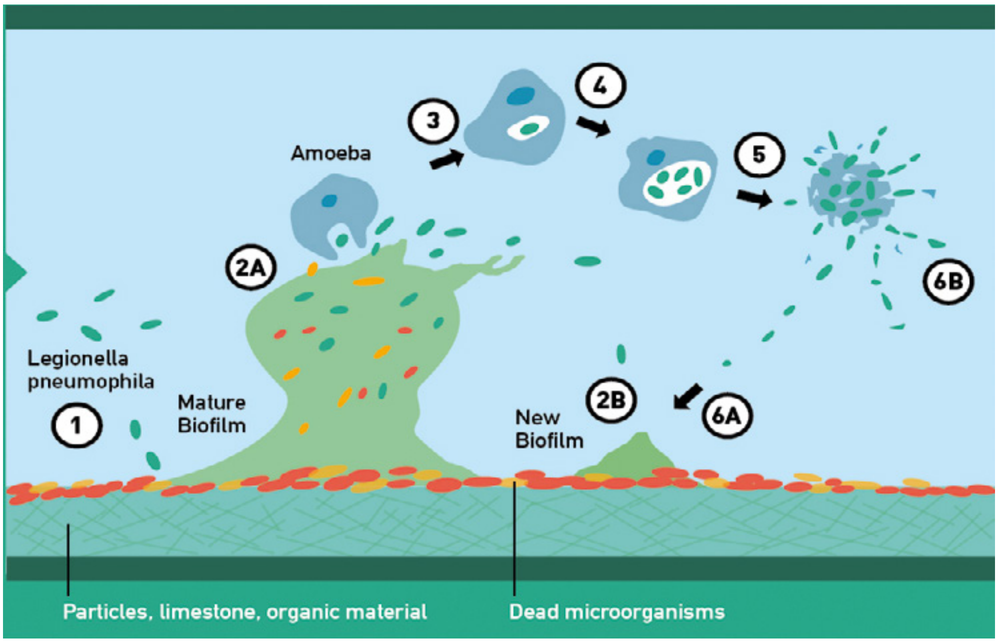
Total bacterial counts are applied in a broad range of Water Safety Plans to control *Legionella*. High bacterial counts indicate poor water quality and an increased risk of developing a *Legionella* problem (Bartram (Ed) et al., 2007) In fact, monitoring overall bacterial levels is far more effective in managing hygiene in water systems than using *Legionella* specific data (Health and Safety Department Dubai, 2010).

Illustration 1



1, Establishment in biofilm; 2A, Amoebae graze on biofilm; 2B, Biofilm spreads and establish growth; 3,Intake of Legionella by Amoebae; 4, Proliferation of Legionella inside Amoebae; 5, Release of Legionella from Amoebae; 6A, New infection of biofilm; 6B, Release into water phase. Graphic kindly provided by Adept Water Technologies

BACTIQUANT®-WATER A RAPID METHOD FOR TOTAL BACTERIA IN WATER SAMPLES:

Bactiquant®-water is a patented field test method for fluorometric detection of a naturally occurring bacterial hydrolase enzyme activity. The hydrolase activity measured using Bactiquant®-water has been shown to correlate with increasing bacterial cell numbers in hot water samples as well as environmental samples (Corfitzen et al., 2006.; Mary Schrock et al.,2011). Hydrolase activity has also been shown to be correlated with endotoxin on surface samples after flooding in buildings (Reeslev et al., 2011). Measuring enzymatic activity is simple and occurs without any extraction procedures. The fluorophore that is released upon hydrolyzation of a fluorogenic enzyme substrate can be measured directly. The fluorescence output is directly correlated with the number of bacteria in the sample. While cultivation of bacteria typically takes several days, hydrolase measurements can be performed on location in minutes.

Bactiquant®-water was included in the guidelines for handling episodes of exceeding limits on drinking water microbiological parameters in drinking water (Danish Ministry of Environment, 2010). The technology has received ETV technology verification by the US-EPA in collaboration with the Batelle standard laboratory, a non-profit research and development organization (Schrock et al. 2011). The technology has been accepted by the Bureau Veritas as part of the verification procedure of ISO 22000 HACCP quality Systems. Recently the technology was included in a 6 million Euro grant funded by the European Commission. The project brings together public and private water operators, research organizations and smart technology providers in a bid to design the drinking water supply of the future (GWI-September 2013 and Seventh Framework Programme 2013).

RISK ASSESMENT IN CRITICAL CARE FACILITIES:

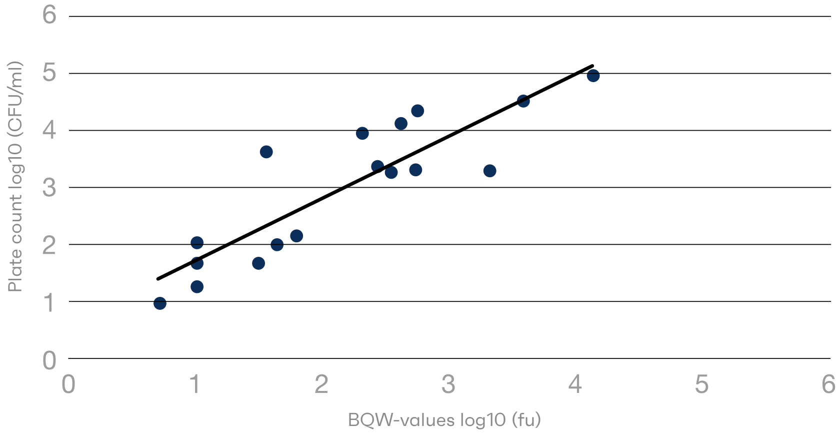
Bactiquant®-water has been used in Critical Care Facilities to identify hotspots of bacterial presence.

In 2001 a two year study was conducted to investigate the feasibility of using ultrafiltration technology to reduce *Legionella* in a Danish Critical Care Facility. In a preliminary survey of the water system, *Legionella* was detected in the system in high concentrations (10^3-10^6 CFU/L). During the investigation, water samples were analyzed in parallel for *Legionella* (Serotype 1 and Serotype 2-14) and for Heterotrophic Plate Counts (HPC), according to DS2402, at four temperatures; 37, 44, 55 and 65°C, and for hydrolase activity using Bactiquant®-water, according to the standard operating procedure specified by the manufacturer's specifications (Bactiquant, Copenhagen, Denmark) All *Legionella* analysis was conducted by an accredited lab (Eurofins, Denmark).

During the study HPC and Hydrolase activity expressed as BQW-value were analyzed in parallel on a subset of hot water samples (n=17).

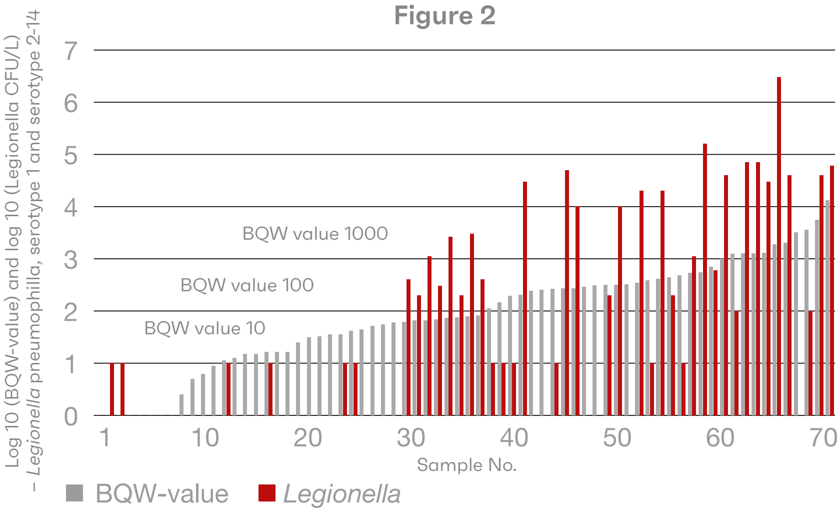
HPC data are shown in figure 1.
The figure shows a good correlation between HPC and BQW-value ($r^2 = 0.75$, $P < 0.0001$)

Figure 1



In order to evaluate the relation between bacterial loads and the occurrence and severity of *Legionella* during the two year study, corresponding BQW-values and *Legionella* concentrations were sorted according to increasing BQW-values (figure 2).

Figure 2



In figure 2, It is readily observed that with increasing BQW-value the risk of finding *Legionella* at high levels also increased. The finding that poor water quality and *Legionella* occurrence and severity are linked is in compliance with many studies showing the importance of availability of complex nutrients and biofilm for the survival and growth of *Legionella* in water systems (Bartram (Ed) et al., 2007).

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In a similar recent investigation, Bactiquant®-water was evaluated as a tool to identify hot spots for health risks in water systems of two major Critical Care Facilities in Copenhagen, Denmark.
The investigation was conducted by the Capital Region of Copenhagen in collaboration with the Ministry of Environment and Food of Denmark.

Water samples were analyzed in parallel for *Legionella* (Serotype 1 and Serotype 2-14) and for hydrolase activity as determined by use of Bactiquant®-water. Statistical analysis of data was performed by Statgroup, Denmark. In this study it was also observed that with increasing BQW-value the risk of finding *Legionella* increased (Figure 3)

Figure 3

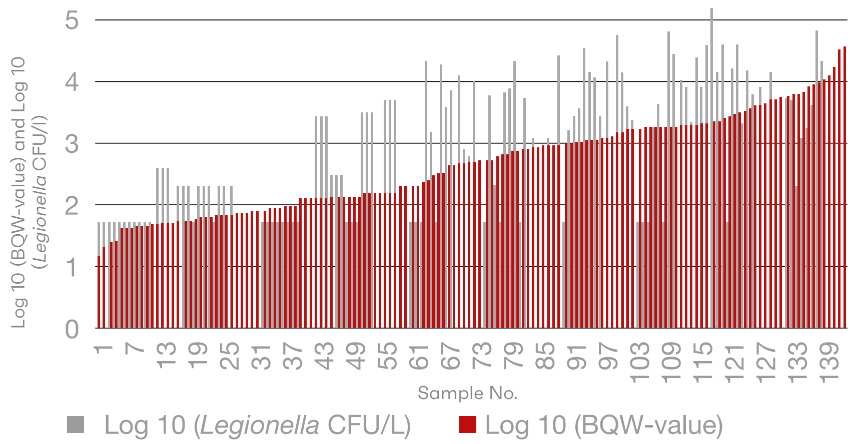
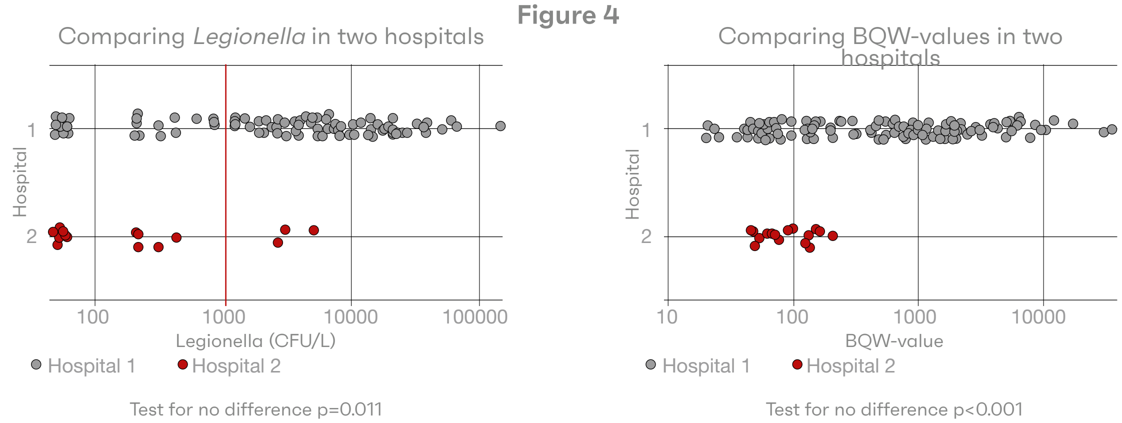


Figure 4 show a box plot of data from the two hospitals. Hospital 1 was characterized by a high proportion of *Legionella* concentrations exceeding 1000 *Legionella* / l (Figure 4a) A similar depiction of BQW-value data (figure 4b) showed BQW-values ranging from 20 to more than 10,000 in hospital 1.

Figure 4

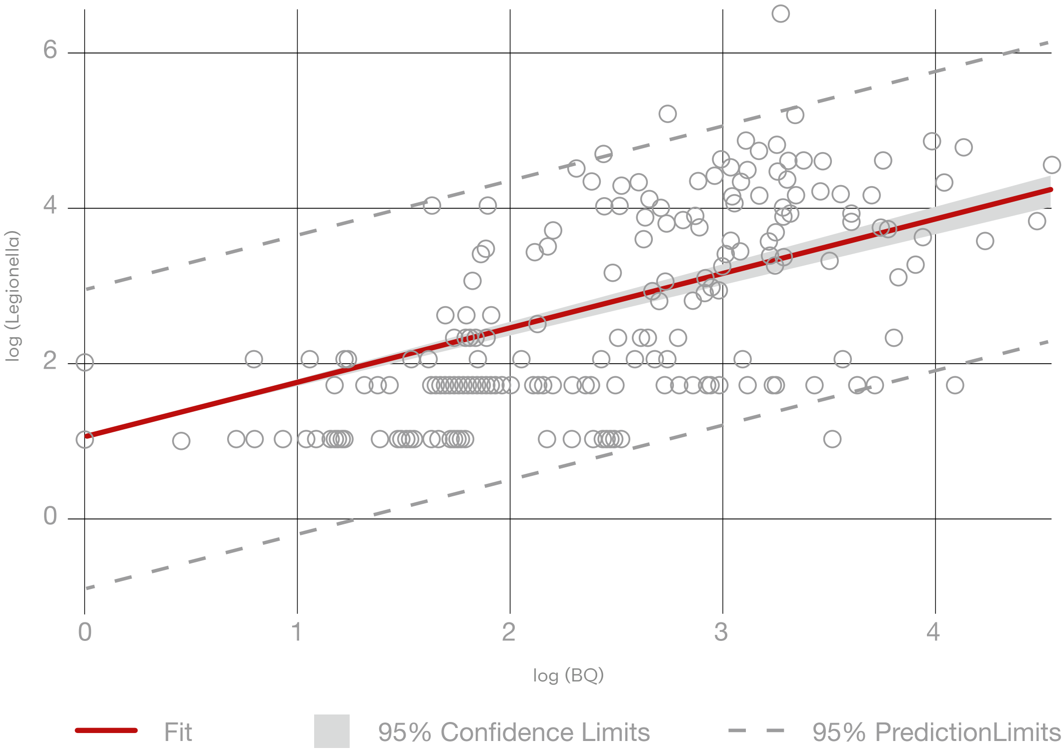


Data from hospital 2 showed few BQW-values higher than 100 and a corresponding low incidence of Legionella concentrations exceeding 1000 Legionella / l.

The data clearly showed a similar degree of dispersion and skewness of BQW-values and *Legionella* concentrations in the two hospitals.

In order to better describe the relation between BQW-values and *Legionella* a linear regression analysis was conducted which also included data from 2001. A fit plot is shown in Figure 5, with log transformed data for BQW-values and *Legionella*.

Figure 5



The data show that with increases in BQW-values there is an increase in *Legionella* concentrations. The figure shows a correlation between *Legionella* concentration and BQW-values (r^2=0,8868).
The proportion of points in the right bottom corner suggests that the relationship is not truly linear. This is not surprising given the fact that water systems characterized by high BQW-values will not always harbor *Legionella*. However, the strength of these data is that despite this fact a clear positive relation is seen between BQW-values and the risk of finding *Legionella* at high concentrations.

Calculations of Positive- and Negative Predictive Values – PPV and NPV statistics - were used to establish a cut off BQW-value for good water system hygiene and low risk of exceeding the limit of 1000 *Legionella* / l.

The statistical analysis included data from the present study and data from 2001.
Table 1 shows that at a cut off value of BQW = 40, no *Legionella* was found exceeding the limit of 1000 *Legionella* / l.

Table 1

BQW cut-off value	True positive rate	False negative rate	True negative rate	Positive predictive value
40	100.0	0.0	25.5	46.6
45	98.9	1.1	31.4	48.4
75	96.6	3.4	50.4	55.8
80	94.4	5.6	53.3	56.8
145	91.0	9.0	66.4	63.8
150	87.6	12.4	67.2	63.4

In conclusion: It is important to control the level of bacteria in a water system. High bacterial loads indicate the presence of growth conditions that are conducive for bacterial proliferation and biofilm formation.

High bacterial loads in water systems give cause for concern for the following reason:

- Risk of finding high levels of endotoxin
- Risk of allergic reactions by exposure to skin and by inhalation of bioaerosols
- Increased risk of *Legionella* contamination
- Smell and discoloration

These studies have shown that Bactiquant®-water provides a rapid comparative and precise report for water quality within the water network. The studies have demonstrated a clear link between poor water quality, as determined by Bactiquant®- water and the risk of finding *Legionella*.

The technology can be used to rapidly identify hotspots of bacterial activity and allows professionals to monitor water system hygiene at regular intervals in real time.

The technology provides a rapid screening tool in post remediation evaluations and for verification of water system hygiene in professional Quality Systems such as ISO 22000/HACCP.

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