

# INVESTIGATION OF *LEGIONELLA* PROLIFERATION IN A HOSPITAL SANITARY WATER NETWORK USING RAPID BIOLOGICAL MAPPING AND HACCP METHODOLOGY

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## INTRODUCTION

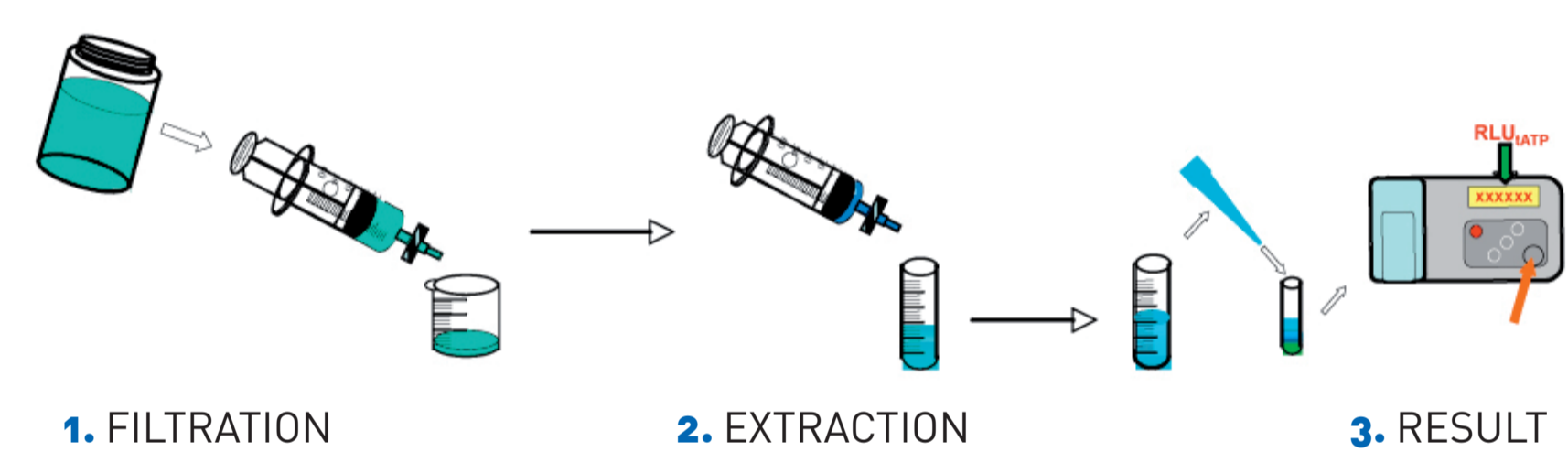
Biological mapping of hospital water networks is an efficient method for identifying critical points for biofilm formation and *Legionella* proliferation in water networks. The use of the HACCP approach to risk assessment aims to identify points where proliferation of both *Legionellae* and *Pseudomonas aeruginosa* occur in the installation. Biological mapping using rapid analytical tools such as 2<sup>nd</sup> generation of ATP-metry allows immediate implementation of corrective or preventive actions together with validation of the efficiency of disinfection, without waiting for long-term culture results.

## SECOND GENERATION OF ATP-METRY: A TOOL FOR RAPID METHODOLOGICAL ANALYSIS OF BIOLOGICAL RISKS

**Quench-Gone Aqueous (QGA™) kit** allows precise quantification of all living microorganisms in water samples and biofilms, through the measurement of intracellular ATP which is present only in viable microorganisms; **both culturable and non culturable**.



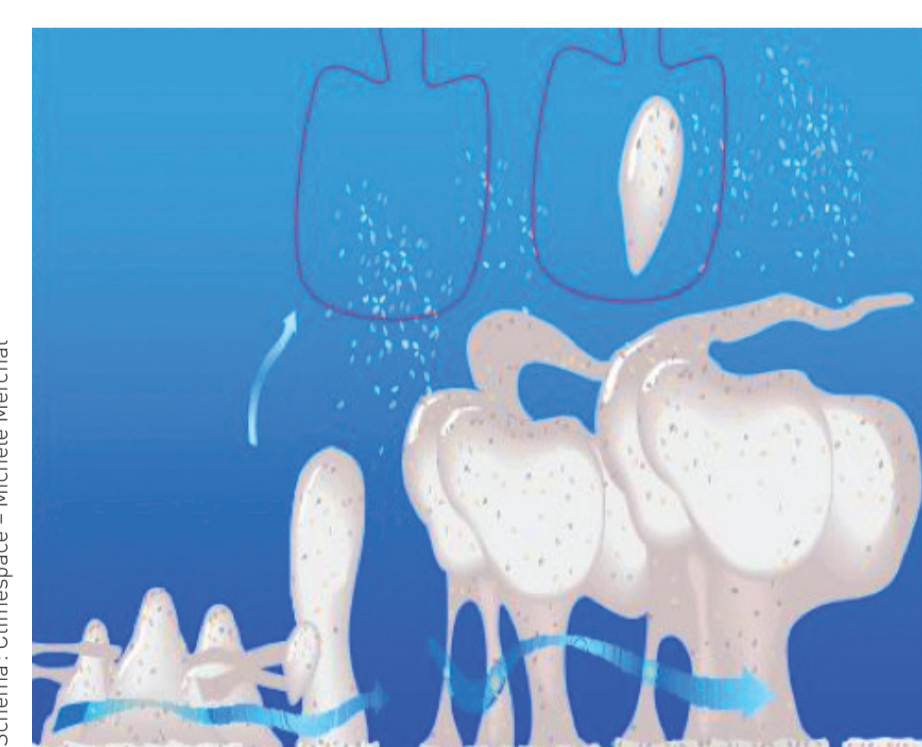
**Protocol:** Intracellular ATP (or cATP) is obtained after filtration of the sample (50ml) and lyses of bacteria retained on the filter for release of ATP. Total time to result is 3min. The quantity of produced light is directly proportional to active biomass: primary results expressed in RLU (Relative Luminescence Unit) are converted in pg ATP/ml or Equivalent microorganisms/ml\* using ATP calibrated standard.



→ **Intracellular ATP is the true indicator of living Total Flora. Thus measured, active biomass includes cultivable, non cultivable or difficult to culture microorganisms.**

→ **A major advantage of using the QGA kit for investigations of water contamination is the ability to produce results in only 3 minutes. A biological investigation of a water network can be completed in 2 hours with immediate on-site interpretation of results.**

## ACTIVE BIOMASS QUANTIFICATION USING QUENCH-GONE AQUEOUS (QGA™) KIT



Elastic biofilm: detachment of flocs of micro-organisms

Intracellular ATP (cATP) is a marker of biomass activity in water installations. 99% of microorganisms are hidden in the biofilm and only 1% of the biomass is in circulating water. Increase of microorganisms in circulating water is representative of high biomass activity in the biofilm. Such an uncontrolled biofilm is characterized by the detachment of flocs of microorganisms – in result, biomass increases in circulating water and is revealed by high cATP values on water samples. Therefore, intracellular ATP can be used for biological risk assessment in sanitary water installations for identifying critical zones for proliferation of pathogenic bacteria, including *Legionella*. High cATP values are representative of an increased risk of the presence of *Legionella* in the installation. On the other side, low cATP values do not necessary mean absence of *Legionella*.

→ **ATP-metry is a tool for rapid methodical analysis of biological risks in water installations.**

\* (1pg/ml of ATP is equivalent to 1000 microorganisms/ml - van Crombrugge J ; Was G (1991) : ATPmethod - Bulletin Fil/IDF 256/1991).

## HACCP METHODOLOGY ADAPTED TO MICROBIAL RISK ASSESMENT IN HOSPITAL SANITARY WATER

**Hazard Analysis Critical Control Point (HACCP)** is a systematic preventive approach to industry that addresses physical, chemical, and biological hazards as a means of prevention rather than finished product inspection. The application of HACCP methodologies seems well adapted for the prevention of *Legionella* risks in sanitary water networks through the systematic analysis of critical zones for proliferation of microorganisms and biofilm formation. Using the QGA kit, the following methodology can be implemented:

1. Establishment of the **simplified architecture** of the sanitary water network.
2. **Microbiological mapping** of the water network for **localization of the microbial proliferation**. Samples are taken from the entire water network – from the inlet of city water up to the end-user points, including hot water productions, water columns, return water etc. **Only a complete sampling schema may lead to an effective risk analysis.** Samples are tested immediately using QGA kit.
3. Identification of the **critical zones** of the installation with **high concentrations of microorganisms**, showing presence of **uncontrolled biofilm growing**. Determination of the parts of the installation showing increased risk for proliferation of *Legionella* and requiring thermal and-chemical cleaning.
4. Optimisation and implementation of **corrective actions**.
5. Immediate evaluation of the **efficiency of the corrective actions** and their **impact on the ecosystem** of the installation – biological mapping after corrective actions.

**Microbiological risks on the installations are reduced if a significant decrease (around 1 logarithm) of global microbial flora measured through Intracellular ATP, is observed.**

## CASE STUDY

A hospital near Paris, composed of four separate buildings, all supplied with hot water from the same production unit, was found to have *Legionella* contamination throughout the water network. All rooms are equipped with thermostatic mixing valves and hot water is delivered at 45°C at terminal points.

Biological mapping of the installation using QGA™ kits was performed on the production unit and in 3 different buildings (Z1,Z2,Z3). *Legionella* contamination was confirmed using the AFNOR NF T 90-431 standard method (Table 1 & table 2).

## RESULTS

Initial Biological Mapping				
Zone	Sample	cATP (pg/ml)	Equivalent Microorganisms/ ml	Legionella pneumophila (CFU/L)
Production	Before Softener	0,45	451	
Production	After Softener	10,58	10582	7500
Production	Return circulation	9,14	9138	
Room Z1	Cold water before mixer	2,16	2163	
Room Z1	Cold water - tap	2,79	2794	
Room Z1	Hot water before mixer	41,95	41951	
Room Z1	Hot water - shower	45,07	45069	3400
Room Z2	Cold water before mixer	3,17	3173	
Room Z2	Cold water - tap	1,12	1118	350
Room Z2	Hot water before mixer	6,12	6121	350
Room Z2	Hot water - shower	11,52	11525	1500
Room Z3	Cold water before mixer	2,79	2790	
Room Z3	Cold water - tap	0,94	944	
Room Z3	Hot water before mixer	80,17	80172	
Room Z3	Hot water - shower	73,38	51289	1200

**Table 1:** Results from Initial Biological Mapping using QGA kit. Results are confirmed using classical culture method for *Legionella pneumophila* quantification.

**Initial Biological Mapping:** The quality of the makeup water was good ( less than 0.45 pgATP/ml or equivalent to approximately 450 microorganisms/ml). Significant microbial contamination (up to 80pgATP/ml or 80 000 Equivalent microorganisms/ml) was detected after the softeners and at the terminal points of the network – showers and taps. *Legionella* presence was later confirmed with concentrations reaching 3 500 CFU/L in the shower heads.

Biological Mapping after disinfection (softener + terminal points)				
Zone	Sample	cATP (pg/ml)	Equivalent Microorganisms/ ml	Legionella pneumophila (CFU/L)
Production	Before Softener			
Production	After Softener	1,05	1050	< 250 nd
Production	Return circulation	1,25	1250	< 250 nd
Room Z1	Cold water before mixer	1,13	1132	< 250 nd
Room Z1	Cold water - tap	2,50	2503	< 250 nd
Room Z1	Hot water before mixer	2,25	2254	< 250 nd
Room Z1	Hot water - shower	1,47	1473	< 250 nd
Room Z2	Cold water before mixer	2,05	2050	< 250 nd
Room Z2	Cold water - tap	0,95	950	< 250 nd
Room Z2	Hot water before mixer	1,52	1520	< 250 nd
Room Z2	Hot water - shower	2,31	2316	< 250 nd
Room Z3	Cold water before mixer	1,58	1580	
Room Z3	Cold water - tap			
Room Z3	Hot water before mixer	1,50	1500	
Room Z3	Hot water - shower	2,05	2050	< 250 nd

**Table 2:** Results from Biological Mapping performed after disinfection of the softener and the terminal points of the installation.

**Confirmation of disinfection efficiency:** An immediate chlorination of the entire water network was implemented (1 mg/l) followed by thermal and chemical disinfection (60°C and 3mg/l of Chlorine) at the terminal points four days later. The softener was also disinfected. A further mapping exercise was carried out and showed a 10-50 fold reduction in viable microbial flora. This was confirmed by negative *Legionella* culture results.

## DISCUSSION

In this hospital the softener and probably another biofilm rich point were responsible for introducing microorganisms and *Legionella* in the hot water installation. The decrease of temperatures below 50°C at terminal points contributes to the installation of *Legionella* at these critical points of the installation. If defaults in thermostatic mixing valves exist, *Legionella* is introduced in cold water. Therefore, a regular control of pressures in hot water and cold water networks is recommended.

In this hospital, the following long-term actions were implemented

- Weakly surveillance of the softener and the rest of the installation using QGA method. If the contamination of the softener occurs at regular basis, maintenance can be adapted and, if necessary, the replacement of the softener can be previewed.
- Correction of the temperature drops in the installation and at the terminal points.
- Elimination of the thermostatic mixing valves and equilibration of the pressure difference between hot water and cold water suppliers.

## CONCLUSION

The principle interest of ATP-metry for health-care establishments is the reactivity that the method is offering for anticipating microbiological drifts in the water installation. Microbial disorders are observed before the classical culture of *Legionella* delivers results. An increase of total microbial flora in the installation shows a decrease in water quality. Additionally, the method offers the possibility to verify the efficiency of water disinfections, on both viable culturable and viable non culturable microorganisms. Immediate corrective actions may be undertaken, even in the absence of *Legionella* culture results. Fragile population present in hospitals benefits from a better protection.