

LEGIONELLA RISK MONITORING IN A HOSPITAL WATER NETWORK through control of bacterial proliferation using a new generation of ATP-metry technology

Jacques Naitychia, Isagua conseil – Veliana Todorova, Aqua-tools – Marc Raymond, Aqua-tools

INTRODUCTION

Deregulated ecosystems are characterized with active biomass present in biofilm, favourable for amoeba, Legionella and other pathogenic bacteria proliferation. For minimizing Legionella risk in hospitals, operators need tools allowing better reactivity to detect progressive degradation of water quality, identify possible Legionella proliferation locations and implement immediate corrective actions.

New generation of bioluminescence method allows precise quantification of viable biomass in water samples and biofilm. Quench-Gone™ Aqueous kit (LuminUltra™ (Canada), supplied in Europe by Aqua-tools) quantifies ATP only from viable microorganisms. The method is used for fast screening of water microbiological quality (results in 6min) and allows detecting in a day time the critical points where biomass proliferates in the biofilm with possible Legionella colonization. Case studies are presented showing biological mapping of hospital water installations where risk analysis could be performed and critical zones identified. The aim of such investigations is to be able to operate immediately when water quality degrades by implementing corrective actions at the most relevant points.

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



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 micro-organisms – in result, biomass increases in circulating water and is revealed by high cATP values on water samples. Therefore, cATP

Elastic biofilm: detachment of flocs of micro-organisms

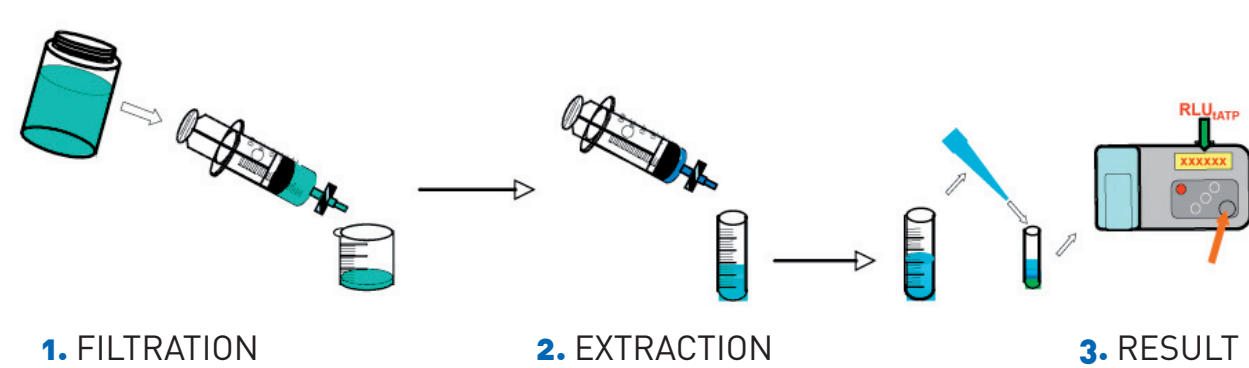
can be used for biological risk assessment in sanitary water installations for identifying critical zones for proliferation of pathogenic bacteria, including Legionella. High cATP values are representative of an increased risk of the presence of Legionella in the installation. On other side, low cATP values do not necessary mean absence of Legionella.

ACTIVE BIOMASS QUANTIFICATION USING QGA™ KIT

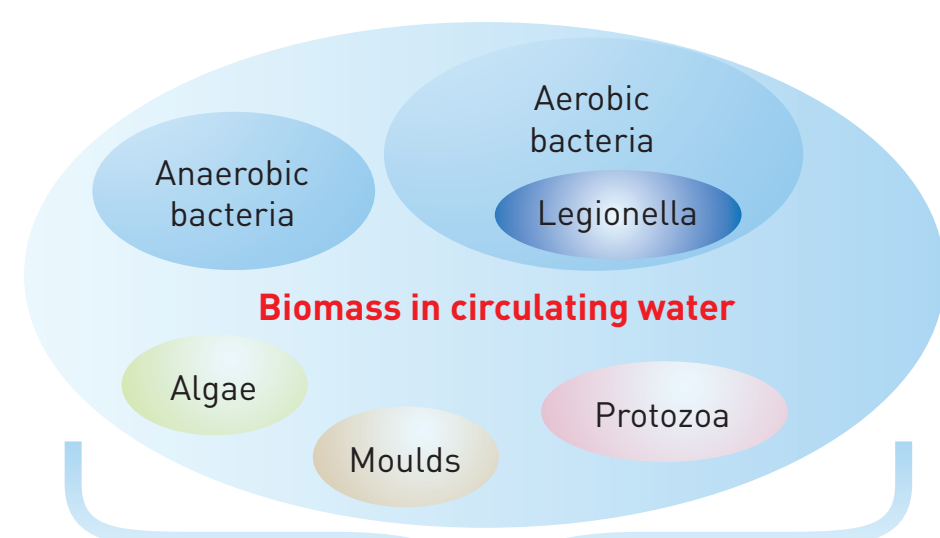
Quench-Gone Aqueous (QGA™) kit allows fast measurement of total viable flora present in water samples through the quantification of intracellular Adenosine Triphosphate (ATP), the energy transporter inside cells.



QGA kits quantify only ATP contained in living microorganisms (Intracellular ATP or cATP). 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 6min. 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.



Intracellular ATP as indicator of total living flora and microbial activity

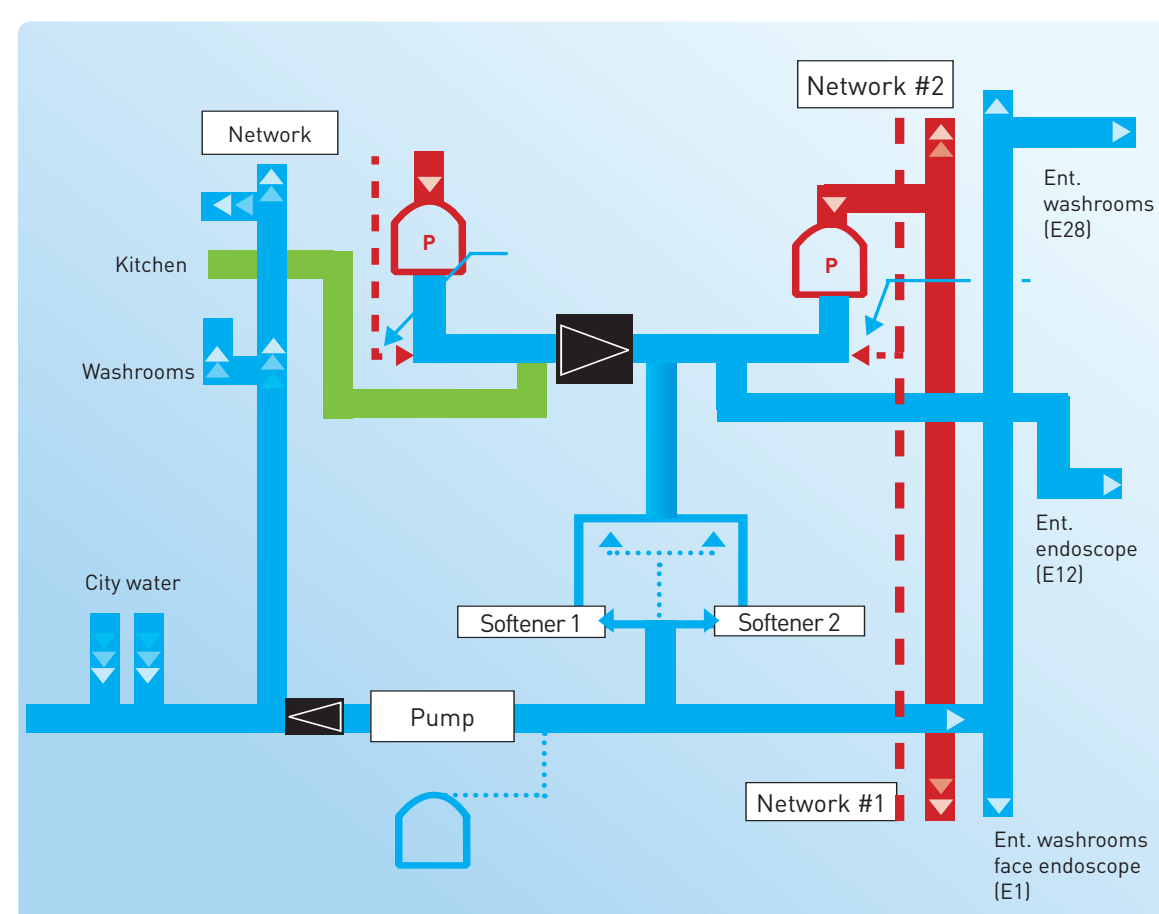
Indicative control cATP values in sanitary water

Process	Sanitary Water
Param.	cATP pg/ml
Good Quality	< 0,5
Preventive Action	0,5 to 10
Corrective Action	> 10

INVESTIGATION OF WATER QUALITY PROBLEMS LINKED TO LEGIONELLA IN HOSPITALS

Investigation procedure in hospitals – step by step:

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/or chemical cleaning
4. Implementation/optimisation of **corrective actions**
5. Immediate evaluation of the **efficiency of the corrective actions** and their **impact on the ecosystem** of the installation



Hospital N°1 :

Previously to ATP mapping, the setting presented regular contaminations by Legionella at 10³ and 10⁴ CFU/L. Biological mapping with QGA was performed at different locations of the building.

SAMPLES		cATP in pg/ml
Water City source	Cold water	0,26
Cold water before tank	Cold water	0,15
Hot water after tank	Hot water	3,55
Roof trussing – column ; 2nd run	Hot water	733,46
1st floor – column ; 2nd run	Hot water	580,55
Ground floor – column ; 2nd run	Hot water	234,68
2nd floor – shower after flexible part ; 1st run	Hot water	361,31
2nd floor – shower upstream flexible part ; 1st run	Hot water	6,95
2nd floor – shower upstream flexible part ; 2nd run	Hot water	3,11
1st floor – shower after flexible part ; 1st run	Hot water	27,76
1st floor – shower upstream flexible part ; 1st run	Hot water	4,17
1st floor – shower upstream flexible part ; 2nd run	Hot water	0,53

* Water is distributed first to the last floor and then is circulating to the lower floors.

Critical cATP values are observed at the column level. Dead legs and zones with established biofilm and active proliferation of biomass probably exist between the production of hot water (tank) and the first point sampled on the column (in the roof trussing).

Significantly high cATP values (361pg/ml and 27pg/ml) are observed after the flexible part of the showers. These results are consistent of biofilm formation, requiring renewing of the equipment.

Hospital n° 2 :

During an investigation in a hospital colonized by Legionella, simultaneous results were obtained by ATP with QGA kit and by culture for Legionella. High active biomass is present at the terminal level of water taps, after flexible, whereas this biomass was much lower upstream. Results were consistent with Legionella culturing.

SAMPLES - COLD WATER			cATP in pg/ml	Legionella concentration (CFU/L)
1st floor – Room 1	Upstream flexible connector of tap	1st run	0,76	350
	Upstream flexible connector of tap	2nd run	0,95	< 250
	Downstream flexible connector of tap	1st run	28,60	1,65.10 ⁴
	Downstream flexible connector of tap	2nd run	1,31	1,75.10 ⁴
2nd floor – Room 2	Upstream flexible connector of tap	1st run	0,54	1,26.10 ⁴
	Upstream flexible connector of tap	2nd run	1,05	600
	Downstream flexible connector of tap	1st run	6,65	1,75.10 ⁴
	Downstream flexible connector of tap	2nd run	2,48	1,05.10 ⁴

*Only the most important values are presented in the table

Change of taps resulted in an immediate decrease of living biomass at the terminal points, associated with Legionella passing under the detection limit. (Legionella was still detected coming probably from biofilm present at the column level, upstream of the taps)

Sterile water was introduced in the 2 different parts of the removed taps – the flexible connector and the terminal, metal, part. cATP values confirmed the hypothesis of an important level of biofilm present in the flexible connector. Moreover, the biofilm was highly colonized by Legionella.

SAMPLES		cATP in pg/ml	Legionella concentration (CFU/100µL)
New taps	1st floor – Room 1: New tap - downstream flexible connector – 1st run	0,08	< 250
	2nd floor – Room 2: New tap - downstream flexible connector – 1st run	0,28	< 250
Removed taps – injection of sterile water	1st floor – Room 1: flexible connector	136,54	840 CFU / 100 µL
	1st floor – Room 1: metal part of the tap	2,95	74 CFU / 100 µL
	2nd floor – Room 2: flexible connector	23,98	64 CFU / 100 µL
	2nd floor – Room 2 : metal part of the tap	0,19	39 CFU / 100 µL

CONCLUSION

Intracellular ATP measurement is a tool well adapted for methodical analysis of biological risks, as it is cost-effective, fast (6min to result) and easy-to-use. After a thorough screening of the sanitary water installation, critical points can be selected and submitted to further analysis for characterization of the biomass by more expensive or more time-requiring methodologies as PCR and culturing of Legionella.

DISCUSSION

Our investigations using active biomass quantification show promising results for fast identification of critical points for Legionella proliferation in sanitary water networks.

The increase of intracellular ATP content measured in water samples in the networks is indicative of proliferation of microorganisms and is a sign of the degradation of water quality. The method is suitable to be used as an early alarm for the prevention of sanitary risks in health-care institutions. The increase of active biomass is representative of a drift in the installation towards unacceptable and risk-associated values. In such conditions of instability and proliferation of total flora, pathogenic bacteria, such as Legionella, develop and colonize the system. The earlier the detection of bacteriological disorders in the installation, the earlier the implementation of corrective actions for decreasing risks associated to proliferation of Legionella.

Quantification of active biomass using ATP-metry allows precise localization of bacterial proliferation zones, followed by the implementation, if necessary, of corrective actions and the validation of their efficiency. All steps, starting from the first mapping of the network up to the validation of the corrective actions, can be performed within hours (whereas traditional methods require 24 to 48 h for identification of the problem).

These observations suggest that monitoring active biomass in sanitary water network using QGA kits may lead to a more efficient management of the installation and early detection and prevention of microbial disorders.

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