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

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INTRODUCTION

Deregulated ecosystems are characterized with active biomass present in biofilm, favorable 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 possibly Legionella proliferation locations and implement immediate corrective actions. New generation of biosensors/microsensor method allows precise quantification of viable biomass in water samples and biofilm. Quench-Gene™ Aquasense kit (Luminvit™ (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 3 min) and allows detecting in a day 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.

1. ATP-METRY: A TOOL FOR RAPID METHODOLOGICAL ANALYSIS OF BIOLICAL RISKS

2. ACTIVE BIOMASS QUANTIFICATION USING QGA™ KIT

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

ATP = luciferin + O₂ → AMP + PP + apurinic acid + LIGHT

QGA kits quantify only ATP contained in living microorganisms (Intracellular ATP, cATP). The increase of intracellular ATP content measured in water samples 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 biological mapping of hospital water installations and is revealed by high cATP values in water samples. Therefore, ATP can be used for biological risk assessment in sanitary water installations for identifying critical zones for proliferation of pathogens, including Legionella. High cATP values are representative of an increased risk of the proliferation of bacteria retained on the filter for release of ATP. Total time to result is 6 min.

3. INVESTIGATION OF WATER QUALITY PROBLEMS LINKED TO LEGIONELLA IN HOSPITALS

Investigation procedures in hospitals - step by step:

1. Establishment of the simplified architecture of the sanitary water network
2. Microbiological mapping of the network for localization of the microbiological proliferation. Samples are taken from the entire water network - from the inlet of city water up to the end use points, including hot water production, 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 growth. Determination of the parts of the installation showing increased risk for proliferation of Legionella and requiring thermal and/or chemical cleaning.
4. Implementation/optimization 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 to 10⁴ CFU/L. Biological mapping with QGA kit was performed at different locations of the building.

### Samples

- **Cold.water**: Hospital N°1: Room 1: 1st fl oor – column; 2nd run Hot water 580,55 CFU/L
- **Hot.water**: Hospital N°1: Room 1: 1st fl oor – column; 2nd run Hot water 361,31 CFU/L
- **Semi-filtered water**: Hospital N°1: Room 1: 1st fl oor – column; 2nd run Hot water 4,17 CFU/L
- **Cooked water**: Hospital N°1: Room 1: 1st fl oor – column; 2nd run Hot water 0,54 CFU/L

### Results

- **Characteristic ATP concentration in sanitary water**: 0,09 CFU/L
- **Corrective Action**: Change of taps resulted in an immediate decrease of living biomass at the terminal level of water taps, after flexible, whereas this biomass was much lower upstream. Results were consistent with Legionella culturing.

### Conclusion

Intracellular ATP measurement is a tool well adapted for methodical analysis of biological risks, as it is cost-effective, fast (6 min 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 this biomass by more expensive or more time-consuming 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 is the indication of proliferation of Legionella 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 uncontrollable 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 intervention of corrective actions for decreasing risks associated by proliferation of Legionella, the more efficient the treatment. The Quench-Gene Aquasense kit (QGA™) allows precise localization of bacterial proliferation. 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.