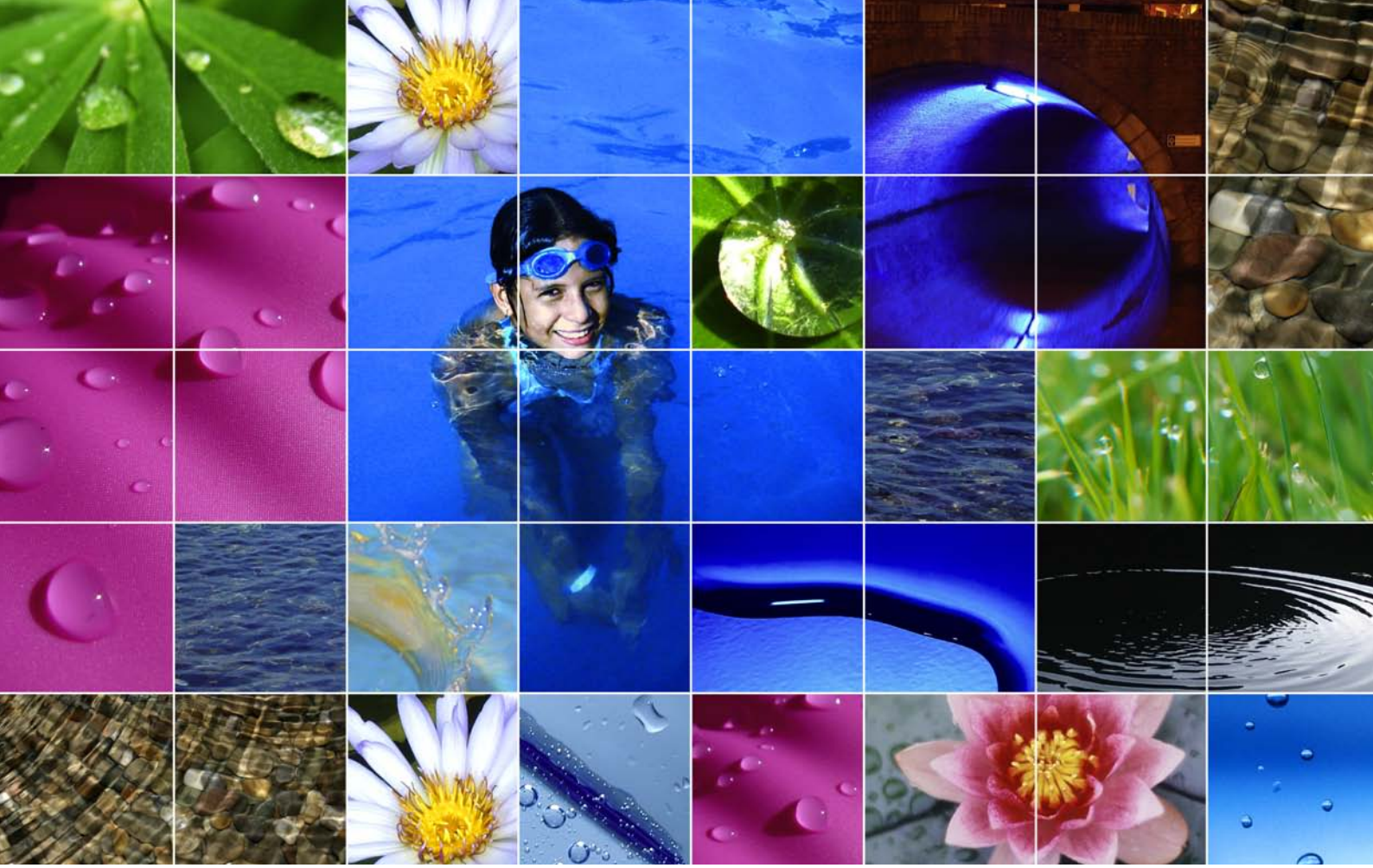


Water testing

→ APPLICATION NOTE #1 - JUNE 2007

Use of Quench-Gone™ Aqueous Kits as a tool to set up Risk Analysis and Routine Surveillance Programs for Sanitary Water and Cooling Tower Systems in Hospitals, Spa.

Laurent Garrelly, GL biocontrol

Jacques Naitychia, Assistance Publique Hôpitaux de Paris

Pierre Allouch, centre Hospitalier de Versailles

Marc Raymond, aqua-tools.com

aqua-tools.com

Water Testing → APPLICATION NOTE #1 - JUNE 2007

INTRODUCTION

Assessment of sanitary risks in the various water networks requires measurement of bacterial contamination. Quench-Gone™ Aqueous test kits provide a fast, reliable, and precise means of measuring the total biomass present in any water sample. Feedback is obtained in only five minutes, allowing the identification of critical control points within the installation and the ability to quickly establish and assess the effectiveness of corrective treatments. This new tool for measurement of active biomass through bioluminescence allows fast follow-up and instantaneous control to fix on one side, effective dosing and the efficiency of a treatment at an installation and, on the other side, to be successful in disinfecting any water network. Quench-Gone Aqueous test kits are a new generation of ATP (Adenosine Triphosphate) measurement, allowing the accurate quantification of intracellular ATP contained within the biomass (in pg/ml).

What is ATP ?

ATP (Adenosine Triphosphate) is the main energy carrier for all living organisms. ATP provides the energy necessary for the biological functions, such as maintenance of the cell, adaptation to environmental changes, consumption of food or reproductive functions. Like any living organism on Earth, microorganisms need ATP to survive and without this molecule, no life could be possible. Therefore, ATP is an essential molecule for microbial life and its concentration is directly dependant on the biomass concentration and the level of stress.

ATP Measurement :

The principle of ATP measurement is based on a bioluminescence technique, in which one photon is produced in the reaction of the enzyme Luciferase and an ATP molecule.

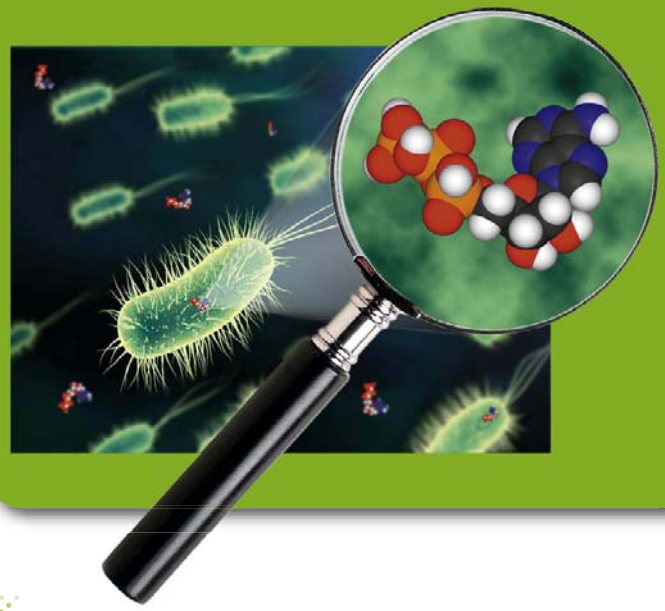
Different types of measurement can be carried out: measurement of total ATP of a sample, the extra-cellular ATP, and also the intra-cellular ATP. These three values are linked through computation of total ATP = intra cellular ATP + extra cellular ATP.

The most discerning parameter for evaluation of microbial quality of water is the intra cellular ATP. This value can be obtained through filtration of a sample and proceeding to lyse of the bacteria retained on a filter to release their ATP for analysis.

FOCUS

→ WHY IS ATP IMPORTANT?

For living functions, any biological cell needs to transform its nutrients (for example, carbohydrates, fats, or proteins) into a usable energy source within the cell. The energy necessary for the different chemical reactions inside the cell is released from the ATP molecule, which during this reaction is reduced into smaller and less energetic forms such as ADP (Adenosine Diphosphate) and AMP (Adenosine Monophosphate). These molecules are then recycled back into ATP as new nutrients are consumed. This process is the central metabolic pathway for any biological cell, including bacteria.



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How is ATP Measured?

The energy contained in ATP is converted into light, using the Luciferin / Luciferase assay. These two molecules are present naturally in the abdomen of Fireflies. Exploitation of this natural phenomenon as part of a chemical test kit gives an instantaneous indicator of the quantity of microorganisms in any type of water or deposit sample.

ATP measurement takes only a few minutes, from sample taking to analysis. ATP is extracted from living cells and prepared to be measured according to the protocol shown below. Dilution of the extract is used to minimize the effects of inhibitors present in the sample.

Intensity of light is measured with a luminometer. The quantity of produced light is directly proportional to the active biomass present in the sample. This reaction is linear over many orders of magnitude, allowing the assay to be used in very clean applications such as drinking water and also in dirty applications such as wastewater.

Surveying Installations using the ATP Measurement

Quench Gone and Total Control test kits developed by **LuminUltra**™ and distributed by **aqua-tools**™ are specifically designed for active biomass measurement in any type of water, including **drinking water**, **hot sanitary water**, **cooling tower water**, and **industrial process water**.
Each kit is designed for specific water types, which can vary in microbial load and the quantity of other contaminants (including suspended solids and dissolved substances).
The active biomass measurement is a simple and fast technology, giving real-time feedback. In addition, the technology is completely portable and only requires one instrument, the Luminometer.

USE

Routinely surveying the total active biomass allows :

Measurement of total active biomass is a valuable continuous survey tool to monitor the microbial evolution of the installation.

→ risk analysis and control
Because ATP is specific to living organisms, the follow-up of an ATP measurement can play a critical role when analysing microbial risks at any location, providing a tool for industrial product quality and improved human health protection.

→ decrease of installation operating costs
Effective monitoring and control of active biomass enables improved cost effectiveness, starting with the cost of injected biocides, up to treatment of waste water.

→ peace of mind
Direct and real-time results allows the detection of contamination as soon as possible and before it's too late. This rapid feedback also allows on-the-spot confirmation of unexpected results – no other biological measurement offers this possibility.

→ reduce environmental impact
Detect residual biomass escaping from factory plant effluents and optimize the use of biocide treatments.

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ACCURACY

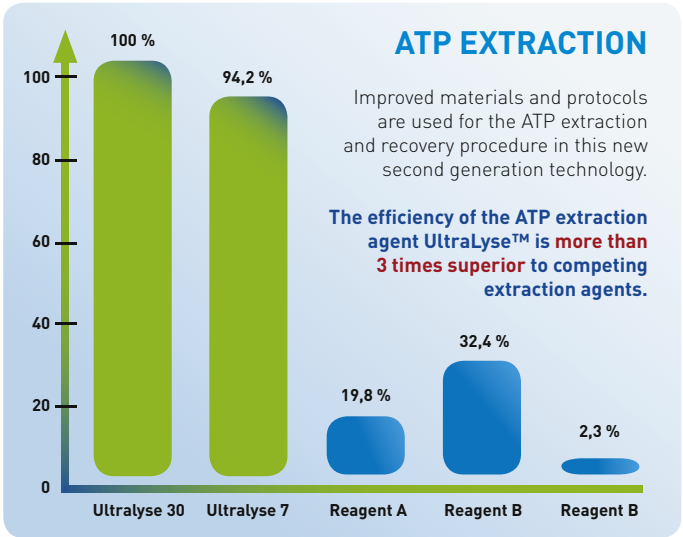
Improved Accuracy over First-Generation ATP Measurements

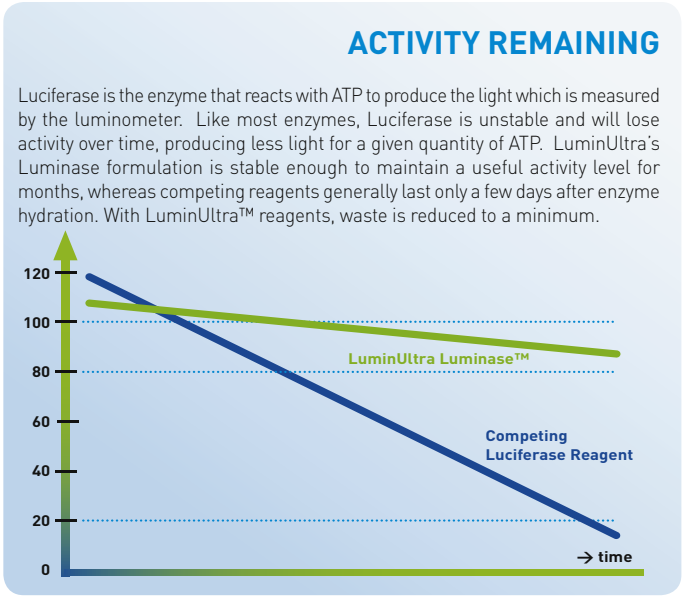
1. The accuracy of the measurement starts with the quality of the sample and the way it is integrated in the analysis. LuminUltra has implemented the following improvements to ensure accurate results during sampling:

- The analysed volume must be great enough to produce a representative indication of the level of contamination. Whereas past products have used 50 or 100 micro liters of sample for integration in the analysis, LuminUltra uses 1 milliliter minimum – the volume of sample can be increased to increase sensitivity of the analysis;
- A pre-treatment using filtration (filter/syringe) and extraction is done on-location, which enables concentration of biomass, elimination of extracellular ATP coming from dead microorganisms and minimizes the effects of inhibitors on the measurement. Following this step, the operator can immediately analyze the extract or store the solution several days for later analysis.

2. First generation ATP-measurement products focus on a more semi-quantitative basis of measurement

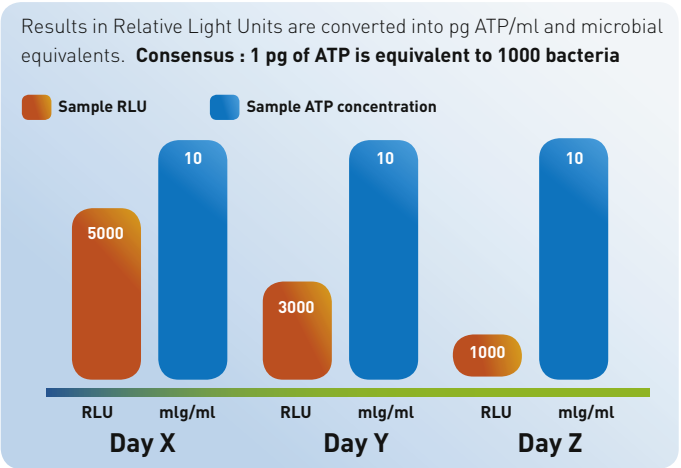
First generation ATP-measurement products focus on a more semi-quantitative basis of measurement, primarily for food hygiene applications. For effective analysis and control of risks, a truly quantitative measurement is preferred, and is especially required in the analysis of water samples. In addition to increasing the volume of sample incorporated into the analysis, and by using a precise volume of sample during the analysis using a micropipettor instead of a semi-quantitative 'dip-stick', LuminUltra has dramatically improved the reagents involved in the analysis:





3. Accuracy of measurement

Most of other suppliers use the Relative Light Unit (RLU) quantity obtained directly from the Luminometer as the indicator of biomass quantity. However, this value will depend on more than just the quantity of biomass – the magnitude of RLU's will depend also on the sensitivity of the luminometer, Luciferase enzyme activity level, and temperature, among others. To eliminate the impact on this 'noise' and maximize the accuracy of measurement, LuminUltra has developed UltraCheck™. UltraCheck is a liquid-stable ATP standard, stable for a several months even at ambient temperature. Utilizing the RLU produced from stable UltraCheck to calibrate sample RLU's not only reduces the noise from other sources, but also enables reliable historical comparisons of an actual ATP concentration from day to day and site to site, regardless of the location, reagent age, and equipment used in the analysis.



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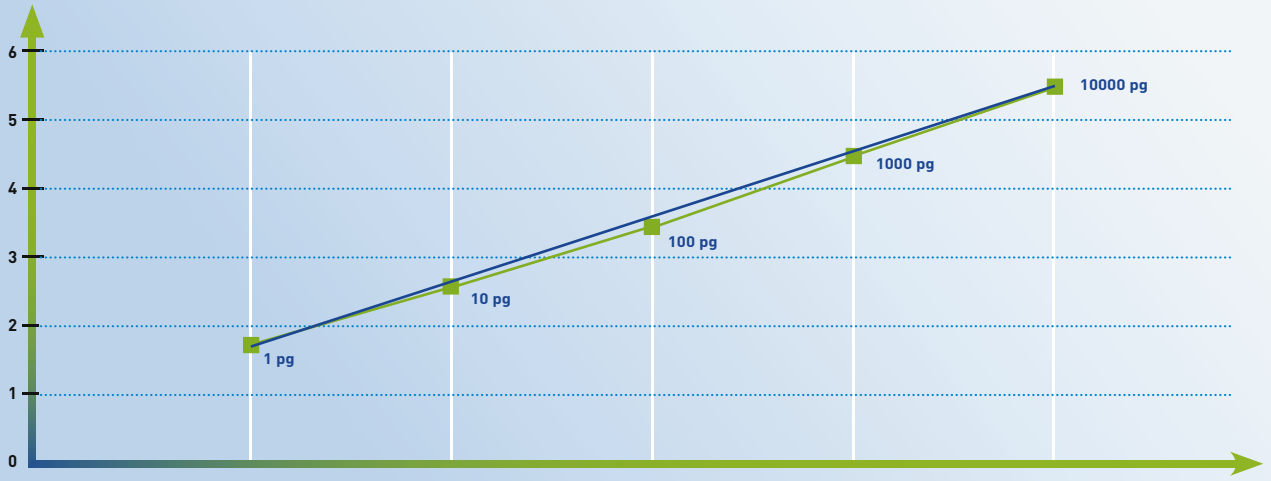
STUDY OF REPEATABILITY OF QUENCH GONE AQUEOUS KITS

Quantity of ATP (Nanograms)	Replicate Measurements (in RLU)					Average	Standard Deviation	CV (%)
	Tube 1	Tube 2	Tube 3	Tube 4	Tube 5			
Blanc	42	49	47	50	47	47	3,082207	**6,6**
0,001	65	76	69	74	75	71,8	4,65832588	**6,5**
0,01	482	384	435	438	394	426,6	39,2020408	**9,2**
0,1	3559	3608	3091	2978	3341	3315,4	278,300737	**8,4**
1	32978	27023	35367	31812	32916	32019,2	3079,62087	**9,6**
10	295940	327283	283946	317055	305068	305858,4	17055,2778	**5,6**

Study carried out by GL biocontrol, Laurent Garelly

QGA REAGENTS – LUMINOMETER KIKKOMAN

Study carried out by GL biocontrol, Laurent Garelly



ATP measurement is a quantitative measurement with a large detection range. Through the use of a series of standards, it is shown that the relationship between RLU (quantity of photons produced) and ATP concentration is linear over 4 orders of magnitude with a low CV.

The quantification limit of the final assay solution is on the order of 1 pg of ATP, with reproducibility (coefficient of variation) of 7%.

When the **ability to concentrate the sample** via filtration is taken into account and given the 1 pg detection limit, it is possible to measure down to 0.2 pg/ml of ATP in a given 'clean' water sample, such as cold sanitary water by filtering 50ml of water. Smaller volumes can be used to measure in waters containing greater quantities of biomass such as cooling water, where concentrations of 100 pg/ml or more are common.

It is important to stress again that **all ATP measurements should be calibrated** to a specific ATP concentration and not simply reported as RLU. It is essential to calibrate the system (reagents and equipment) before starting any measurement. This can be done with an ATP standard corresponding to 1 ng/ml. (UltraCheck 1). For each luminometer a low value limit and an upper value limit provide **verification of the linearity** of the system and its detection range.

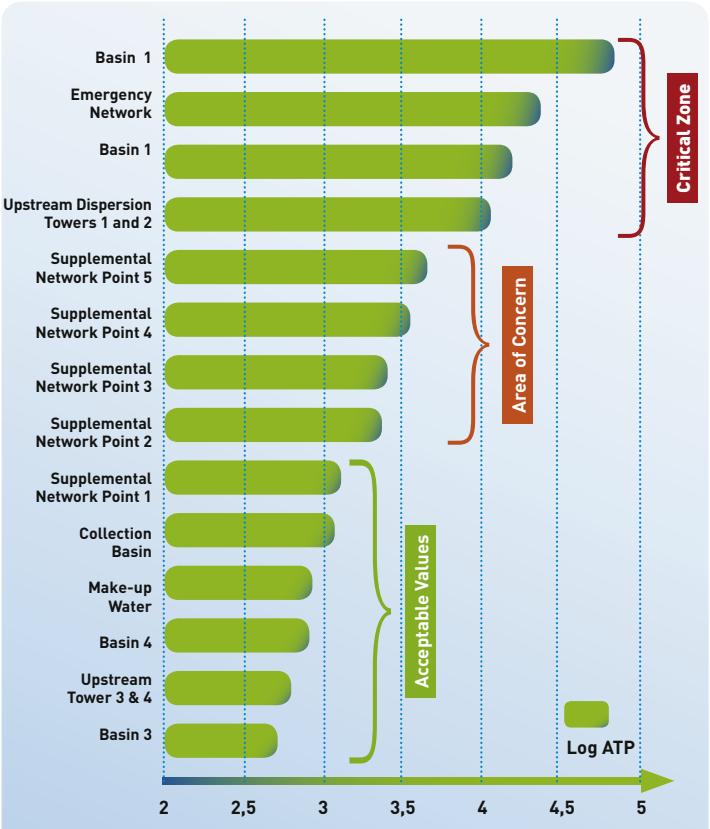
USE

How can I use ATP Measurements to Survey an Installation?

1. Define the Control Points using Methodical Risk Analysis

Measuring the planktonic intracellular ATP in a variety of locations throughout a given water system allows an assessment of the level of risk through the system. Depending on the size and complexity of the system, it is common to identify up to 10 critical control points for routine monitoring.

Example : Survey of Intracellular ATP in cooling tower water system.



In this example, a total of 14 points were measured for intracellular ATP concentration within a cooling tower water system. Obtaining these results and comparing them enables the risk at each measurement point to be assessed to identify areas of concern.

2. Identify Critical Points and Set up a Routine Test Program

Moving forward, a routine testing schedule can be established for the identified critical control points. Major critical points can be followed-up at higher rate than others to gain improved control, which is an effective approach given the low cost associated with this test and that a biomass growth rate of more than 0.3 log (i.e. growth of 100%) has to be considered as significant and has to be explained or at least controlled in the early stages of detection for effective control.

3. Install Preventive and/or Corrective Actions

Actions to maintain effective control decided and driven by the Methodical Risk Analysis Committee are meant to reduce the risks for installations. These actions can most often be followed-up by ATP measurement. For example, a chemical cleaning action implemented to reduce the biomass fixed on the surface of an installation will reduce the fixed (sessile) biomass, but will temporarily increase the planktonic biomass. Kinetics of these two actions should be assessed in order to optimize the cleaning event by purging the lines or to inject biocide to neutralize the dropped bacteria. Using ATP measurements to assess these effects is a perfect rapid and direct solution to optimize this approach.

4. Survey a Balanced and Stable Installation

According to the NF T 90- 471 guidelines, a specific analysis has to be done every month in a cooling tower network. However, ecosystem excesses sometimes appear very quickly and the situation will be all the more difficult to reverse if not detected as it occurs. Therefore, early detection of any starting change will be an advantage, and a more frequent measure of total biomass using ATP monitoring perfectly fits to this approach.

5. Perform Audit of locations

Example: Using ATP measurements to define the control points using the Methodical Risk Analysis and compensating actions.

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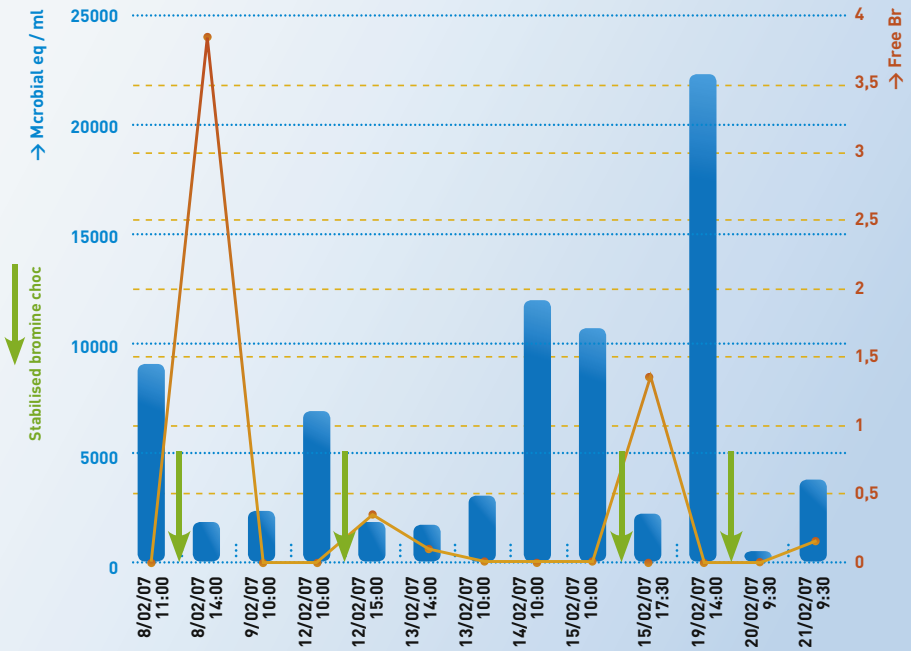
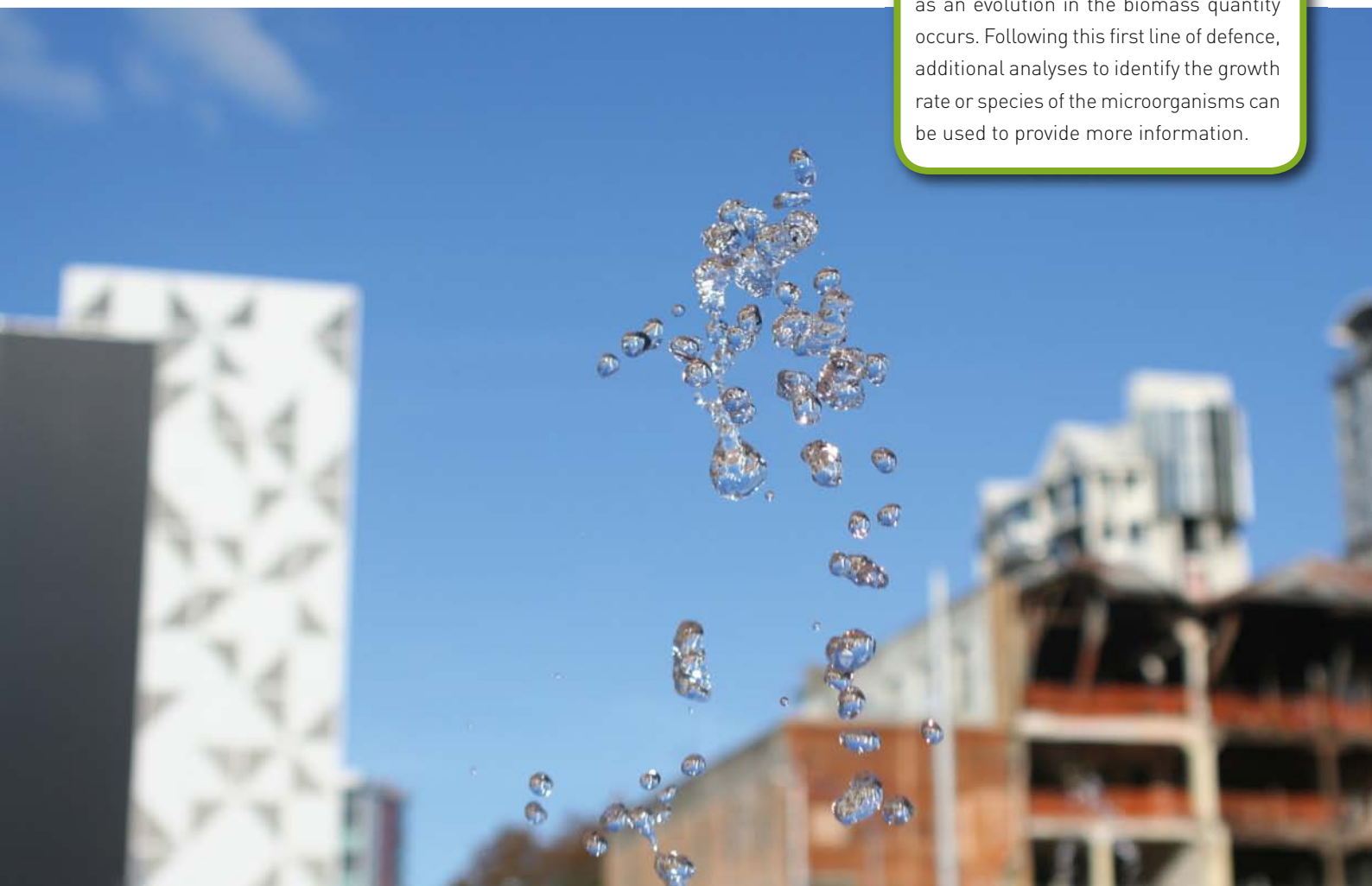
ACTIVE BIOMASS BY ATP : T1 / T2



Altis, France- Carine Magdo

CONCLUSIONS

ATP measurements are the best indicator for operational monitoring and tracking corrective and preventive actions of risks associated with active microorganisms in any water application. From source water to drinking water and even industrial process water, measurement of ATP is the first line of defence as part of a strategy for microbiological risk analysis and controls (according to the philosophy of AMDEC, HACCP, or similar methods). ATP measurements can be used as a daily surveillance tool for active biomass concentration in any water system. Measuring total biomass on a frequent basis will provide an early warning of impending issues, which will help operators to supervise the critical points of the installation in order to be proactive as soon as an evolution in the biomass quantity occurs. Following this first line of defence, additional analyses to identify the growth rate or species of the microorganisms can be used to provide more information.



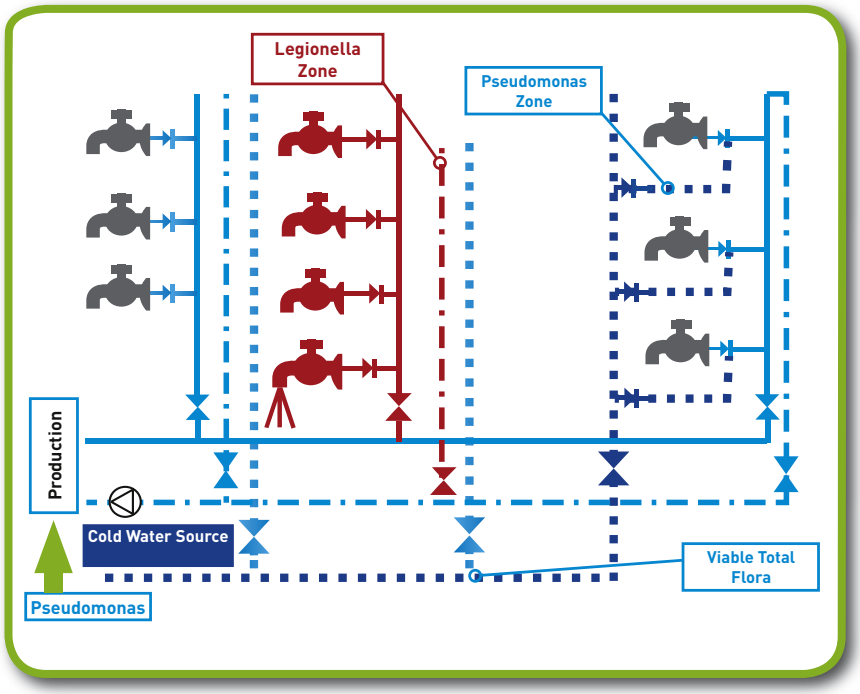
Water Testing → APPLICATION NOTE #1 - JUNE 2007

CASE STUDY #1

Localized contamination in a cold water system

Jacques NAITYCHIA (Public Assistance Paris Hospitals)

Problem:
Over the course of several months, a hospital had observed a persistent contamination in the cold water circuit supplying the endoscope room. A survey of the system using ATP measurements was completed at different points throughout the system to determine if contamination was localized or if the entire system had excessive amounts of bacteria. The preference would be to identify critical points and take localized corrective actions rather than undertaking a disinfection of the global network. A global disinfection approach would be more difficult considering the consistent need of water throughout the hospital for equipment and patients, to say nothing of the elevated cost. However, without completing a survey this approach would be the only one available.

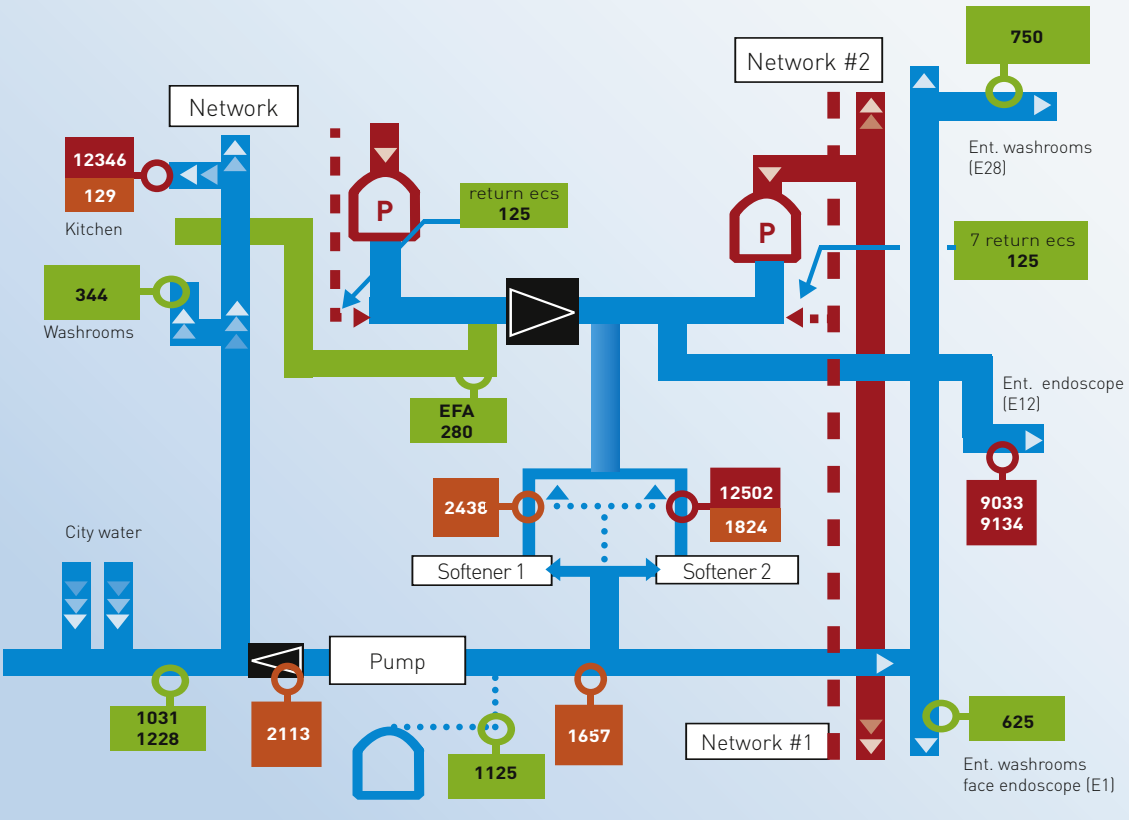


LOCATION OF CONTAMINATION IN COLD WATER SYSTEM

Results are expressed in equivalents of microorganisms per ml (1 microbial equivalent = 1 fg of ATP). **Green** coloured values represent acceptable biomass levels. **Orange** ones identify areas which may have impending issues and require preventive action. **Red** ones represent values that indicate the need for immediate corrective action.

Following these initial tests, the operator can take measures to correct microbiological risks. Two actions were taken to help reduce microbial contamination within the system:

1 Treatment of Water Softener #2 was performed.
2. Thermal shock located at Kitchen sample point.



Water Testing → APPLICATION NOTE #1 - JUNE 2007

After having established a plan to sample for the cold and hot water distribution lines, a sampling plan was established as follows: cold water inlet, auxiliary equipment (including pressure reducers and water softeners), main inlet pipes supplying both distribution networks (#1 and #2) and finally the local inlet pipes supplying the endoscope rooms.

The first series of ATP measurement analysis, made in one half day, clearly shows that the water softener #2 had a major build-up of biomass, which appeared to be responsible for the contamination of the water line supplying the endoscope room. This piping is directly connected behind this softener. In addition, the reference point of the kitchen is qualified as contaminated.

Following the corrective actions, measurements were repeated and are shown as the second number at each point on the diagram. After disinfection of the softener the results come back to an acceptable level. The piping supplying the endoscope room has developed a biofilm resistant to a chemical decontamination. A softener has to be installed in the room of endoscopes to liberate the network.

The two analyses on the return sealing taps of hot sanitary water clearly show that the disinfection action (through reverse current circulation of chlorinated water at 60°C) is efficient in removal of legionella. It should be noted that the colour of the water coming from the stagnant sealing taps (the source of the contamination) was of the colour of coffee.

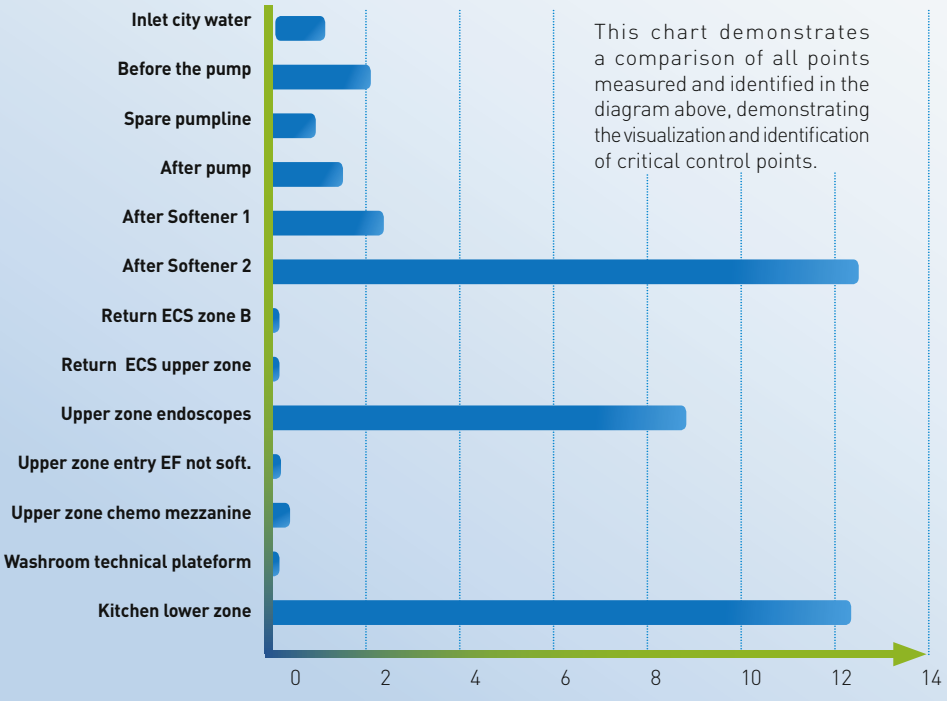
OUTCOME

Jacques Naitychia

Using two sets of measurements before and after corrective actions, ATP measurements using the Quench-Gone Aqueous technique facilitate quick identification of localized contamination, decision on corrective action, and immediate follow-up of the results without wasting time. After this step, specific bacterial analyses are established to verify regulatory compliance.

In conclusion, this case study demonstrates the utility of Quench-Gone Aqueous as a tool to assess and follow-up total biomass evolution in a water network on a proactive basis. Routine total biomass measurements provide an effective tool for water system protection and allow **prevention of problems instead of recovery from problems!**

ATP MEASUREMENT ANALYSE PER DAY : MARCH 15TH 2007



Recommended Volumes for Analysis

Based on multiple evaluations, LuminUltra recommends the following sample volumes for filtration during a QGA analysis, depending on the type of water and biocide used if any:

Source	Sample Volume
Potable Water	50 – 100 ml
Cooling water	10 – 50 ml
Recycle Water	5 – 25 ml
Fresh Water	5 – 25 ml
Ultra-pure water	100+ ml

Volumes can be adjusted to increase sensitivity if necessary

Recommendations :

QGA can be used for real-time supervision of clean water installations to optimize the installed microbiological control programs. The table below gives an idea of the target, but any operator should keep these values only as information, each water plant being different.

Process	Param.	Good Qualtiy	Preventive Action	Corrective Action
Potable Water	cATP (pg/ml)	< 0,5	0,5 à 10	> 10
Cooling Water (Non-Oxidizing Biocides	cATP (pg/ml)	<100	100 à 1.000	> 1.000
Cooling Water (Oxidizing Biocides)*	cATP (pg/ml)	<10	10 à 100	>100
Surface or Recycle water	cATP (pg/ml)	< 5	5 à 100	> 100

Including chlorine, bromine, peroxyde, etc.

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CASE STUDY #2

Versailles Hospital Complex

Doctor Pierre ALLOUCH

Study carried out in December 2006 by Nelly Breton, Hygiene Operator)

Over the course of a few days the following analyses were performed as part of this case study to assess biological contamination throughout the hospital water network:

Water types analyzed:

Water for standard care: 49 spots

Water for food use: 12 spots

Water bacterially controlled: 27 spots

Softened water for dialyser: 27 spots

Cooling tower water: 3 spots

Reference method:

Filtration on celluloid ester membrane

Research for aerobic invigorated flore, Pseudomonas aeruginosa and coliformes :

Culture on jelly medium (TCS, coliformes selectives, cetrimide)

Delay of 24 to 72 h

Action taken if 1 pathogen is detected and/or numeration >100 UFC. By this determination, a total of 11% of the points in the network are contaminated.

Reference Method Results

Water	Absence of alert	Alert for Pathogen presence	Alert for aerobic invigorated flore
for standard care	100	5	3
for food use	50	0	1
bacterially controlled	150	2	6
water for dialyser	6	3	12
cooling tower water	5	0	13
Total	**311**	**10**	**35**

QGA™ Test Kit results

Water	< 1pg ATP/ml	> 1pg ATP/ml
for standard care	32	16
for food use	12	1
bacterially controlled	27	0
water for dialyser	0	9
cooling tower water	1	2
Total	**72**	**28**

QGA™ :

Action taken if concentration > 1 pg ATP/ml of biomass is present. By this determination, a total of 28% of the points in the network are contaminated.

TIME REQUIRED TO OBTAIN RESULTS	ATP MEASUREMENT	BACTERIAL CULTURE		
	Network Water	Water for standard care	Water for food use	Water bacterially controlled
On site sampling	5 min	5 min	5 min	5 min
Recording of figures	1 min	1 min	1 min	1 min
Preparation - maintenance of instruments	2 min	5 à 10 min		
Filtration- transfer of assay	2 min	3 min	3 min	2 min
Reading - Interpretation				
IF RESULT is NEGATIVE	< 1 min	< 1 min		
IF RESULT is POSITIVE		5 to 10 min		
Time required to obtain results	**Real Time**	**D+3**		

CONCLUSION OF STUDY

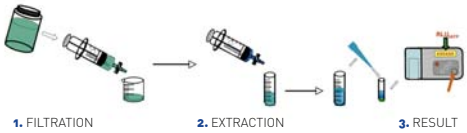
By Doctor Allouch (Hospital of Versailles)

QGA™ kits are :

- A sensitive method to determine biomass
- Easy to operate
- Fast
- Less expensive than bacterial cultures;
- Well-suited for prevention of biological contamination.

Kits QG™ (Quench Gone)

Principle: Quench-Gone™ kits are designed for low-solids water applications and use filtration to separate biomass from the sample. Filtration makes it possible to concentrate the active biomass and to separate the extracellular ATP and 90 to 95% of the inhibitors present in the sample. UltraLyse™ makes it possible to lyse the bacteria retained on the filter and subsequently extract the intracellular ATP (cATP™). This parameter is an indicator of total microbiological load in the water. UltraLyse™ has been optimize to maximize ATP extraction and has been shown to extract more than 3 times the ATP recovered by other commercial extraction agents.
Quench-Gone™ kits measure the total active biomass in all the types of water using a single analysis. The results from a Quench-Gone™ measurement can be converted into Microbial Equivalents (i.e. the total number of microorganisms per ml of sample).



QGA™ (Quench-Gone™ Aqueous)

Presentation : QGA™ is optimized to detect microbial equivalents ranging between 500 and 667,000,000 per ml (0.5 to 6.7x10^5 pg ATP/ml). The volume of sample processed can be adapted (i.e. increased) to amplify the sensitivity, which allows can allow users to detect 0.2 pg/ml (200 Microbial Equivalents per ml) or lower for water known as «clean».
Strategy of use: Use QGA™ to quickly survey a process to and determine the critical control points; subsequently make follow-ups at the critical points to maintain effective control at the installation. QGA™ can also be used to quickly troubleshoot a process.
■ **TYPES OF WATER:** surface water, ultrapure water, papermaking, ground water, cooling water, water disinfection, potable water, fire water, wastewater, etc.

Catalog N°	Process up to...	Including
QGA-100	**100 samples**	12.5 ml Luminase™ (Luciferase Enzyme Reagent) 2.5 ml UltraCheck™ 1 (1ng/ml ATP Standard) 125 ml UltraLyse™ 7 (ATP Extraction Agent) 1000 ml UltraLute™ (tATP Dilution Buffer)
QGprep	**100 samples**	100 x 60 ml Syringes w/ Luer-Lok Tip ; 100 x Syringe Filters, 25mm

QGO™ (Quench-Gone™ Organic)

Presentation : QGO™ is optimized to detect microbial equivalents ranging between 500 and 67,000,000 per ml (0.5 to 6.7x10^4 pg ATP/ml). QGO™ is designed for water-based solutions containing organic contaminants, such as organic particles or petrochemicals.
■ **TYPES OF WATER:** metalworking fluids, recycle papermaking streams, pulp processing streams, wastewater, etc.

Catalog N°	Process up to...	Including
QGO-100	**100 samples**	12.5mL Luminase™ (Luciferase Enzyme Reagent) 2.5ml UltraCheck™ 1 (1ng/ml ATP Standard) 500ml LumiClean™ (ATP Extraction Agent) 100ml UltraBuff™ (ATP Dilution Buffer)
QGprep	**100 samples**	100 x 60ml Syringes w/ Luer-Lok Tip ; 100 x Syringe Filters, 25mm

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