Use of Quench Gone Aqueous™ kits as a tool for implementing methodical risk analysis and surveillance programs in water networks

ATP Monitoring Technology for Microbial Growth Control
Introduction: What is ATP-metry?

- Method for **simple and fast** measurement of ATP (Adénosine TriPhosphate) concentration in the cell

- **1st generation of ATP tests**
  - Used in food industry for the estimation of microbial contamination after surface cleaning.
    - Qualitative estimation of the level of microbial contamination,
    - \(< 50 \text{ RLU}, >100 \text{ RLU, } 50<\text{X}>100\)

- **2nd generation of ATP tests**
  - Developed for all water matrices
    - Quantitative tools indicating precisely the number of organisms
    - Results delivered in Picogram of ATP /ml and microbial equivalent
Introduction: What is ATP-metry?

• Adenosine Triphosphate (ATP):
  – Issued from the Glycolyse reaction in living cells,
    • ATP forming
  – ATP is the energy carrier of the cell

• ATP = «fuel» of any living cell
  – ATP is the energy source for many chemical reactions
  – Provides the energy necessary for all biological functions
    • Adaptation to the environment,
    • Food consumption,
    • Reproduction functions

• ATP is an essential molecule for microbial life
  – Aerobic, anaerobic, and anoxic bacteria
How does the ATP technique work?

• The technology is based on **bioluminescence** principle

• An enzymatic reaction transforms ATP in Light, in the presence of Luciférine / Luciférase complex

  \[
  \text{ATP} + \text{luciferin} + \text{O}_2 \xrightarrow{\text{Mg}^{++}, \text{luciferase}} \text{AMP} + \text{PP}_i + \text{oxyluciferin} + \text{LIGHT}
  \]

• A mathematical formula allows to transform the intensity of Light measured in ATP concentration
How to use ATP measurements? : QUALITY APPROACH

- Fast identification of the critical points of the network
- Establishment of threshold guidelines according to the water quality requirements of the network
- Immediate interpretation of results and validation of the treatment procedure within 1 hour
- Immediate corrective actions implementation if required
- Progressive decrease of water quality
Different forms of ATP

- Intracellular ATP
  - cATP: Active biomass / Living organisms
  - dATP: Dead organisms or Dying organisms
- Total ATP
  - tATP: intracellular ATP + extracellular ATP
- Biomass stress
  - BSI: dATP / tATP

- ATP measurement is the best indicator of biological activity of total flora
  - Viable cultivable bacteria
  - Viable non cultivable bacteria
  - Bacteria difficult to grow
THE 1ST MONITORING TECHNOLOGY FOR CONTROL OF ACTIVE BIOMASS IN REAL TIME

- Improve treatment efficiency on viable and cultivable or not cultivable micro organisms
- Optimize biocide dosing programs
- Validate treatment and its frequency
- Eliminate pathogenic organisms

- Improve potable water quality
- Optimize reclaimed water treatment
- Control of disinfection process
- Protect public health

- Improve process stability
- Optimize biomass health
- Monitor raw wastewater toxicity
- Reduce operating costs

COOLING WATER AND SANITARY WATER NETWORKS

POTABLE WATER AND PROCESS WATER

BIOLOGICAL WASTEWATER TREATMENT

aqua-tools.com
### Test Kit Contents

<table>
<thead>
<tr>
<th>Included in QGA Kits</th>
<th>Storage Temp.</th>
<th>Shelf Life</th>
</tr>
</thead>
<tbody>
<tr>
<td>Luminase</td>
<td>4°C</td>
<td>3 mo.</td>
</tr>
<tr>
<td>Luciferase Enzyme Reagent</td>
<td></td>
<td></td>
</tr>
<tr>
<td>UltraCheck 1</td>
<td>20°C</td>
<td>6 mo.</td>
</tr>
<tr>
<td>1 ng/mL ATP Standard</td>
<td></td>
<td></td>
</tr>
<tr>
<td>UltraLyse 7</td>
<td>20°C</td>
<td>12 mo.</td>
</tr>
<tr>
<td>ATP Extraction Reagent</td>
<td></td>
<td></td>
</tr>
<tr>
<td>UltraLute</td>
<td>20°C</td>
<td>12 mo.</td>
</tr>
<tr>
<td>tATP Dilution Buffer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>60mL Syringe w/ Luer-Lok</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>25mm Syringe Filters w/ Luer-Lok</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

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**Quench-Gone™**

**NATURAL WATER**

**ULTRAPURE WATER**

**SANITARY WATER**

**COOLING TOWER WATER**

**FIRE WATER**

**RECYCLE WATER**
# Performances of QGA™ kits

Validation according to AFNOR XP T 90 210

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Performances of the kits</th>
<th>Benefits of QGA™ kits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quantification limit</td>
<td>0.1 pg ATP / ml = 100 microbial equivalents (EqB)</td>
<td>The quantification is possible at very low levels of contamination</td>
</tr>
<tr>
<td>Linearity of the quantification</td>
<td>5 log of amplitude&lt;br&gt;From 0.1 to 10000 pg/ml&lt;br&gt;Equivalent to 100 to 10^6 EqB</td>
<td>The kits can be used on water with different bacteriological quality</td>
</tr>
<tr>
<td>Results variation</td>
<td>&lt; 10%</td>
<td>Comparable results whatever the day of analysis</td>
</tr>
<tr>
<td>Recovery of ATP extraction</td>
<td>&gt; 90%</td>
<td>Establishement of threshold guidelines according to the water type</td>
</tr>
<tr>
<td>Repeatability of the measure</td>
<td>On 5 series with 5 contamination levels: CV = 10%</td>
<td>Instant results</td>
</tr>
<tr>
<td>Time to results</td>
<td>10 minutes</td>
<td>Flexibility of analysis</td>
</tr>
<tr>
<td>Stability of ATP extracts</td>
<td>Up to 7 days</td>
<td></td>
</tr>
</tbody>
</table>

- 1 E. coli = 1 fg ATP
- 1 pg ATP = 1000 microbial equivalents (International consensus)
Sample Volume Guidelines

Based on experience, LuminUltra recommends the following volumes be used in step 1 for samples processed using QGA test kits:

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Recommended Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drinking water</td>
<td>50 – 100 mL</td>
</tr>
<tr>
<td>Cooling water</td>
<td>10 – 50 mL</td>
</tr>
<tr>
<td>Reuse water</td>
<td>5 – 25 mL</td>
</tr>
<tr>
<td>Fresh water</td>
<td>5 – 25 mL</td>
</tr>
<tr>
<td>Ultra pure water</td>
<td>100+/ mL</td>
</tr>
</tbody>
</table>

Volumes can be adjusted to increase or decrease analysis sensitivity as required.

Kit Use and Interpretation

QGA is designed for measurement of biomass in ‘clean’ water samples. Measure cATP on each sample, convert using UltraCheck results, and then calculate ATP concentration.

Although each process or application is different, use these guidelines to establish your control program.

<table>
<thead>
<tr>
<th>Process</th>
<th>Parameter</th>
<th>Good Control</th>
<th>Preventive Action Required</th>
<th>Corrective Action Required</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drinking Water</td>
<td>cATP (pg/mL)</td>
<td>&lt; 0.5</td>
<td>0.5 to 10</td>
<td>&gt; 10</td>
</tr>
<tr>
<td>Cooling Water (Non-Oxidizing Biocides)</td>
<td>cATP (pg/mL)</td>
<td>&lt; 100</td>
<td>0 to 1,000</td>
<td>&gt; 1,000</td>
</tr>
<tr>
<td>Cooling Water (Oxidizing Biocides)*</td>
<td>cATP (pg/mL)</td>
<td>&lt; 10</td>
<td>10 to 100</td>
<td>&gt; 100</td>
</tr>
<tr>
<td>Fresh or Reuse Water</td>
<td>cATP (pg/mL)</td>
<td>&lt; 5</td>
<td>5 to 100</td>
<td>&gt; 100</td>
</tr>
</tbody>
</table>

*Includes chlorine, bromine, peroxide, etc.
Use: Biocide Treatment Follow-up

Biomasse active par ATP : GF CTF et FS

H2O2/Ag (après 3 mois de traitement continu)

Action corrective

Action préventive

Bonne qualité

eq microbien/

1,E+06
1,E+05
1,E+04
1,E+03
1,E+02

date

Br libre = 0

GF2 GF6 GF8 GF9 GF10 GF11 GF12 GF14 GF13
Use: Biocide Treatment Follow-up

**Biomasse active par ATP : T1/T2**

<table>
<thead>
<tr>
<th>Date</th>
<th>Time</th>
<th>eq microbien/ml</th>
<th>oxydant (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8/2/07</td>
<td>11:00</td>
<td>10000</td>
<td></td>
</tr>
<tr>
<td>8/2/07</td>
<td>14:00</td>
<td>5000</td>
<td></td>
</tr>
<tr>
<td>9/2/07</td>
<td>10:00</td>
<td>15000</td>
<td></td>
</tr>
<tr>
<td>12/2/07</td>
<td>10:00</td>
<td>20000</td>
<td></td>
</tr>
<tr>
<td>13/2/07</td>
<td>10:00</td>
<td>25000</td>
<td></td>
</tr>
<tr>
<td>13/2/07</td>
<td>14:00</td>
<td>20000</td>
<td></td>
</tr>
<tr>
<td>14/2/07</td>
<td>10:00</td>
<td>25000</td>
<td></td>
</tr>
<tr>
<td>15/2/07</td>
<td>10:00</td>
<td>20000</td>
<td></td>
</tr>
<tr>
<td>15/2/07</td>
<td>17:30</td>
<td>25000</td>
<td></td>
</tr>
<tr>
<td>19/2/07</td>
<td>14:00</td>
<td>20000</td>
<td></td>
</tr>
<tr>
<td>20/2/07</td>
<td>9:30</td>
<td>10000</td>
<td></td>
</tr>
<tr>
<td>21/2/07</td>
<td>9:30</td>
<td>5000</td>
<td></td>
</tr>
</tbody>
</table>

**Choc brome libre stabilisé**
Comparison between culture (CFU), PCR (GU) and Biomass quantification (EqB)

GF14: legionella spp (UG/L) - Biomasse active (eq microbien/L)
Results

Cooling towers surveillance program:

- Effective tool for monitoring
- No interference between oxidizing biocides and bioluminescence
- ATP and Legionella PCR results have the same trend
- Concordance between water network knowledge, field observations and operations
- Concordance between operating mode and efficiency of biocide treatment

Baseline threshold for each cooling tower network:

- **Accepted level = 10^3** microbial equivalents/ml (1 ATP pg/ml)
  → bacterial flora under control, efficient treatment
- **Alarm = 10^5** microbial equivalents/ml (100 ATP pg/ml)
  → unstable process, risk to reach high culture levels
Benefits of biomass quantification

- Instant indication of microbial level = Tool for instant follow-up of the process
- Analysis to be performed on site or in a laboratory
- Fast method – 10 minutes / sample
- Low cost

The approach can be used in the following situations:

- Cost reduction
- Reduction of global reagents consumption
- Reduction of global water consumption
- Consideration of environmental and sanitary issues of the installation

Complementary tool to other microbiological methods
Biomass quantification: what for?

- Auto-control
  → Reactivity: Results in 10min (10 days per culture/48h per PCR)
  → Anticipation of process derivation
- Biocide efficiency on VC and VNC organisms
  → Improve efficiency of treatments
  → Optimize biocide concentration
  → Validate efficiency of disinfection
- Identification of pollutions
- Economical issue: cost reduction
  → Stop total flora analysis

Industrial mode: reliability, reactivity, low cost

cATP: Implement regular follow-up 3 times per week for monitoring of CT treatment process
Evolution de la [ATP] dans l'installation

[ATP] en pg/mL

Points contrôlés:
- Eau puit
- Après pompe
- Avant tour
- Après tour
- Eau décarbonaté
- Après machine
Case study: Enertherm

End user
Facility Manager
Water treatment company
(Expert laboratory)

Put the facility under Microbiology Risk Controls

Biosecurity
Survey of the installation using QGA™ kit

• Have a Picture :
  – quantification of the Biomass during one month, on different point of sampling
  – Define correct base line, in terms of concentration of ATP

• Survey :
  – Study the evolution of the concentration of biomass after the day choc treatment
Survey of the installation using QGA™ kit

• Points of sampling

  – Seine River
  – Seine rive water after step filtration (sand)
  – Tank (with chlorine treatment)
  – Groupe 1 make up process
  – Groupe 2 make up process
  – Groupe 3 make up process
  – Groupe 4 make up process
  – Groupe 5 make up process
  – Groupe 6 make up process

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<th>Param.</th>
<th>Good Quality</th>
<th>Preventive Action</th>
<th>Corrective Action</th>
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</thead>
<tbody>
<tr>
<td>Potable Water</td>
<td>cATP (pg/ml)</td>
<td>&lt; 0.5</td>
<td>0.5 à 10</td>
<td>&gt; 10</td>
</tr>
<tr>
<td>Cooling Water (Non-Oxidizing Biocides)</td>
<td>cATP (pg/ml)</td>
<td>&lt; 100</td>
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<tr>
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<td>cATP (pg/ml)</td>
<td>&lt; 10</td>
<td>10 à 100</td>
<td>&gt; 100</td>
</tr>
<tr>
<td>Surface or Recycle water</td>
<td>cATP (pg/ml)</td>
<td>&lt; 5</td>
<td>5 à 100</td>
<td>&gt; 100</td>
</tr>
</tbody>
</table>

*including chlorine, bromine, peroxide, etc.
Survey of the installation using QGA™ kit

**Tuesday Morning**

**Tuesday PM after isothiazolone traitement**

**Wednesday**

**Thursday**

**Friday**

**Tuesday PM after isothiazolone traitement**

cATP™ - Intracellular ATP

[Charts showing ATP levels on different dates and times]
Sanitary water microbial contamination
Contamination of the network: Should all the network be disinfected?
**Case study 1 – Health care institution (1):**

<table>
<thead>
<tr>
<th>Réseau de distribution Intérrne</th>
<th>Réseau de distribution Intérrne</th>
</tr>
</thead>
<tbody>
<tr>
<td>03 – Sortie surpresseur – Niv S/Sol</td>
<td>04 – Puisage Circulation – Niv +4</td>
</tr>
<tr>
<td>05 – Poste de soins USIP – – Niv +4</td>
<td>06 – Lavabo Ch. X – Niv +4</td>
</tr>
<tr>
<td>07 – Lave bassin Ch. X – Niv +4</td>
<td>08 à 14 – Prélèvements Ch. XX – Niv +4</td>
</tr>
<tr>
<td>08 – Lave Bassin Aval Flexible</td>
<td>09 – Douche</td>
</tr>
<tr>
<td>10 – Sortie colonne en GT, en amont Lavabo et Lave mains</td>
<td>11 – Sortie colonne en GT, en amont du Lave bassin et WC</td>
</tr>
<tr>
<td>12 – Sortie colonne en GT, en amont de la douche</td>
<td>13 – Douche après désinfection thermique 60°C 30min.</td>
</tr>
<tr>
<td>14 – Lave bassin Amont Flexible</td>
<td></td>
</tr>
</tbody>
</table>
### Case study 1 – Health care institution (2):

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Value RLU</td>
<td>9</td>
<td>3</td>
<td>28</td>
<td>4</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Value ATP en pg/ml</td>
<td>0.2</td>
<td>0.1</td>
<td>0.7</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Equivalent Bact/ml</td>
<td>213</td>
<td>71</td>
<td>663</td>
<td>95</td>
<td>95</td>
<td>71</td>
</tr>
<tr>
<td>Log bact/ml</td>
<td>2.33</td>
<td>1.85</td>
<td>2.62</td>
<td>1.98</td>
<td>1.98</td>
<td>1.85</td>
</tr>
<tr>
<td>UG spp/l</td>
<td>&lt; LD</td>
<td>&lt; LD</td>
<td>&lt; LD</td>
<td>&lt; LD</td>
<td>&lt; LD</td>
<td>&lt; LD</td>
</tr>
<tr>
<td>Log UG spp/l</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UG pneumo /l</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>08 à 14 – Prélèvements Ch. XX – Niv +4</th>
<th>08 – Lave Bassin Aval Flexible</th>
<th>09 – Douche</th>
<th>10 – Sortie colonne en GT, en amont Lavabo et Lave mains</th>
<th>11 – Sortie colonne en GT, en amont du Lave bassin et WC</th>
<th>12 – Sortie colonne en GT, en amont de la douche</th>
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<th>14 – Lave bassin Amont Flexible</th>
</tr>
</thead>
<tbody>
<tr>
<td>Value RLU</td>
<td>4571</td>
<td>1700</td>
<td>21</td>
<td>18</td>
<td>NF</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>Value ATP en pg/ml</td>
<td>108.2</td>
<td>40.3</td>
<td>0.5</td>
<td>0.4</td>
<td>NF</td>
<td>0.1</td>
<td>0.2</td>
</tr>
<tr>
<td>Equivalent Bact/ml</td>
<td>108228</td>
<td>40251</td>
<td>497</td>
<td>426</td>
<td>NF</td>
<td>95</td>
<td>189</td>
</tr>
<tr>
<td>Log bact/ml</td>
<td>5.03</td>
<td>4.60</td>
<td>2.70</td>
<td>2.63</td>
<td>NF</td>
<td>1.98</td>
<td>2.28</td>
</tr>
<tr>
<td>UG spp/l</td>
<td>130000</td>
<td>NF</td>
<td>NF</td>
<td>NF</td>
<td>NF</td>
<td>NF</td>
<td>NF</td>
</tr>
<tr>
<td>Log UG spp/l</td>
<td>5.11</td>
<td>NF</td>
<td>NF</td>
<td>NF</td>
<td>NF</td>
<td>NF</td>
<td>NF</td>
</tr>
<tr>
<td>UG pneumo /l</td>
<td>&lt; LD</td>
<td>NF</td>
<td>NF</td>
<td>NF</td>
<td>NF</td>
<td>NF</td>
<td>NF</td>
</tr>
</tbody>
</table>

The contamination is principally at the extremities (oxygen and mixed water). The contamination increases in flexible parts of the network (biofilm, contact with)
Case study 2 - Health care institution (1)

Study performed thanks to the advices of Jacques Naitychia
Case study 2 - Health care institution (2)

**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!

Study performed thanks to the advices of Jacques Naitychia
Biomass evolution (Kit QGA (en pg ATP/ml))

Analyses on a shower system before and after the flexible part

- Tap
  - 0.3 pg/ml CW
  - 2.4 pg/ml HW > 50°C

- Hand mixer

- 9.1 pg/ml (30°C)
- 5.3 pg/ml (55°C - 2 mn)
- 2.8 pg/ml (55°C - 30 mn)

After thermal & chemical disinfection

How to disinfect the tap water system?: inject Chlor in hot water during the disinfection phase
Biofilm in the flexible part of a shower

Prof. Dr. med. Martin Exner M.D., Ph.D.
Chairman and Director
Institute for Hygiene and Public Health
WHO CC
University of Bonn
Case History – ATP: Spedali Civili Brescia

Spedali Civili de Brescia 27 juillet 2007
Case History – ATP: Spedali Civili Brescia

Spedali Civili de Brescia 27 juillet 2007

ATP [pg/ml] 

1- Récirculation Reseau Eau Chaud
2- pharmacie - labo - Froid
3- pharmacie - labo - Chaud
4- rianimation - salle bon. Froid
5- rianimation - salle bon. Chaud
6- Cuisines - rinçage legumes Froid
7- Cuisines - rinçage legumes Chaud
8- Chir. Plast. Chambre 13-14 Chaud
9- Infectives - Cuisine - Chaud
10- Med. Nucl.-Salle visite Chaud

sanipur.com
Case History - ATP: Ospedali Riuniti Bergamo

![ATP Measurement Graph]

Ospedali Riuniti Bergamo 13 Aout 2007

ATP [pg/ml]

<table>
<thead>
<tr>
<th>Location</th>
<th>ATP (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC sx Trauma 2p toilete</td>
<td></td>
</tr>
<tr>
<td>CC sx Neurochir 2p smaltitoio</td>
<td></td>
</tr>
<tr>
<td>CC cx farmacia pt bagno donne</td>
<td></td>
</tr>
<tr>
<td>CC dx Med 2p toilette interno</td>
<td></td>
</tr>
<tr>
<td>CC dx Med 2p toilette fire</td>
<td></td>
</tr>
<tr>
<td>CC cx Emontol 2p bagno pubbl</td>
<td></td>
</tr>
</tbody>
</table>

Aqua fredda centrale termica
General conclusions

• Why use QGA™ kit?
  – Monitor bacteriological disorders in real-time in sanitary installations without the need of a laboratory station
  – Autonomy allowing generation of significant time saving.

• What do the results bring to the user?
  – Implement a strategy turned to the installation. Does a treatment have to be applied? At which moment action has to take place? In case of a decided treatment, verification of discerning disinfection has to be done: chemical, thermal or thermo-chemical choice (at d and d+20)

• How to interpret the obtained results?
  – Guidelines of any installation are driven by the evolution of the results. This method doesn’t substitute the regulation controls.

• Conclusions:
  – Allows to better understand the reaction between bacteria and hydraulics, with regard to the different events
  – Allows to act almost immediately, to adapt and identify actions
  – Is a maintenance tool for any actor in water applications facilities to make them aware and train themselves
  – Allows them to achieve autonomy
They trust us:

- Water treatment companies
  - DUPUY SAS
  - AQUAPROX

- Engineering services and audits
  - ANTAGUA
  - CLIMESPACE
  - Biocontrol

- Utilities management
  - Ondeo Svez
  - Veolia Environnement
  - Cofatec
  - Institut Curie

- Industries
  - ArcelorMittal
  - EDF
  - Enertherm
  - Air Liquide
  - Altis

- Health-care institutions
  - Institut Curie

- Laboratoires
  - Eurofins
Special thanks to:

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