GLBIOCONTROL

RAPID MICROBIAL DIAGNOSTIC

OPERATING MODE DENDRIDIAG® SW

Measuring the quantity of bacteria by ATP-metry

- DRINKING WATER -

CONTENT

GL BIOCONTROL

GL BIOCONTROL specializes in environmental risk management and is an expert in sanitary engineering along with biological monitoring of water and surfaces.

Our clients are environment professionals: industrialists, industry operators, water treatment companies, laboratories, study design engineer and public authorities.

We have several skills including: risk assessments, research and development, analysis, product development and professional training. Through these areas of expertise, GL BIOCONTROL:

- **Develops** risk management tools (ATP-metry kits for total flora quantification, DNA extraction purification kits, real time PCR amplification kits…).
- **Uses** methodologies and innovative tools to study the microbial world (qPCR, NGS, ATP-metry…).
- **Studies** ecosystems to anticipate and prevent public health risks, in particular linked to *Legionella* and *Pseudomonas* genus (risk assessment, microbiology diagnostics, ATP cartography…).
- **Advises** water sector professionals on how to manage their facilities in order to reduce public health risks as well as improve the environmental footprint (water, treatment products and energy conservation).
- **Trains** environmental professionals on microbiological risk management and laboratory techniques.

All our products are made in France.

What is quantitative ATP-metry?

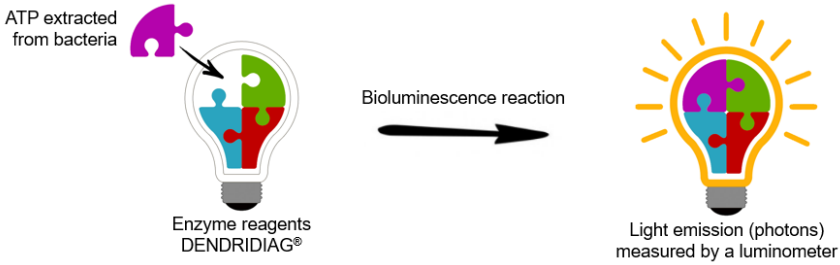
ATP-metry is a microbiological technique that evaluates the **overall bacterial load** of a water sample in less than **2 minutes**.

It is based on the detection of ATP molecules (energy-carrying molecule) which is found only in living cells:

« Any trace of ATP proves the existence of living organisms. »

▶ Watch the video

Once extracted from bacteria, the ATP reacts with our reagents, in particular with the luciferase enzyme which comes from the firefly's tail. The amount of light emitted is **proportional to the quantity of microorganisms**. It is measured by a luminometer.

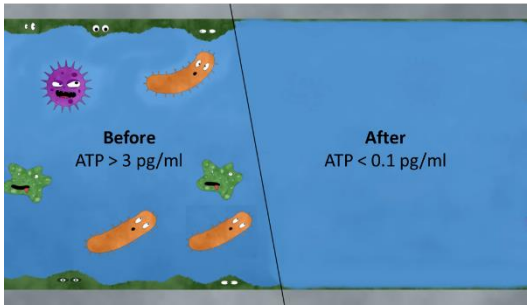


Each measurement becomes **quantitative** because of the addition of a standard which takes into account environmental factors (temperature, pH, inhibitors…).

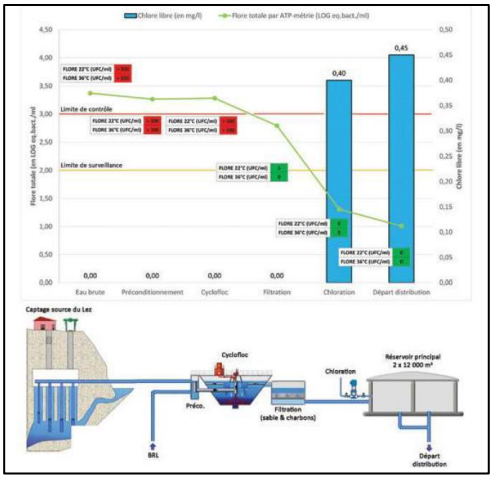
1 pg of ATP ≈ 1 000 bacteria

This easy and reliable on-site analysis is a great **decision support tool**. It allows you to validate disinfections on-site, to look for critical points, to handle crisis situations…

Example of cleaning & disinfection

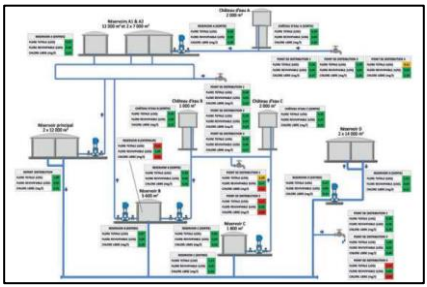


Applications



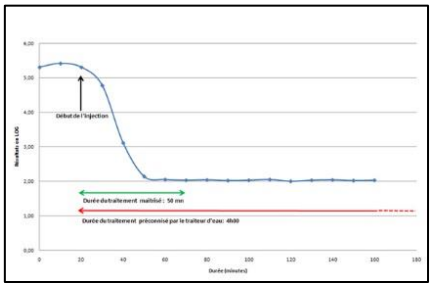
Monitoring of the drinking water treatment plants, from the source to the water conveyance:

- Mapping of a treatment plant and assessment of the treatment efficacy at each step.
- On-site validation of the potabilization process (contact time…)
- Immediate adjustment of treatments in response to the source's variations.
- Evaluation in real time of the sand filters' bacterial load to adapt maintenance procedures.



Monitoring of the water supply network:

- Quickly identify a degradation of the water quality.
- Detect critical points.
- Anticipate microbiological shifts by defining warning thresholds.
- Management of pipe flushing and chlorination systems.



Assessment of the treatment efficiency:

- Return to service of a system (tank, treatment plant…) without delay.
- Validate the cleaning and disinfection procedure immediately after maintenance operation or in crisis situations.

Quantitative ATP-metry is a validation and support decision tool to better manage risks in accordance with WSPs.

Equipment needed

Reagent kit (60 tests)*

Product	Quantity
EXTRACTANT dropper	1
STANDARD dropper	1
Aluminum bag of 10 test tubes **LUMITUBE**	6

Consumable kit (60 tests)

Product	Quantity
Single-use sterile 60ml sampling containers	60
Single-use filtration syringes of 50ml	60
Single-use filters 0.45µm pore size	60

Equipment

Product	Quantity
Luminometer KIKKOMAN PD30 or equivalent	1
Tube holder for PD30	1

** The reagents can be stored at room temperature and in the dark for 3 months. To ensure an optimum conservation over 1 year, we advise you to keep them refrigerated (2 - 8°C).*

Protocol

Sampling

Collect samples in accordance with the good practices. First, flame the sampling outlet. Then, let the water run to flush the sampling line (at least 30 sec for cold water and until you get a stable temperature for hot water).
Place the sampling container under the water flow without modifying the flow rate. Once filled, remove the sampling container, and then stop the water flow. The analysis must be performed within an hour after sampling. If not, collect the water in a sample bottle with sodium thiosulfate, and store in the fridge for 48h maximum.

Installation

Take the **EXTRACTANT** and **STANDARD** droppers out as well as a **LUMITUBE** bag.

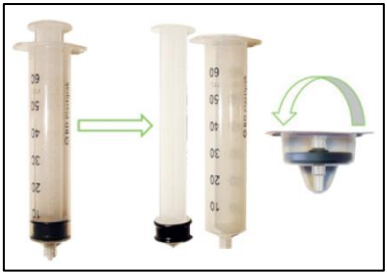
Make sure they are at room temperature (>18°C) before use.

Prepare the plastic consumables (sampling container, syringe and filter), and turn on the luminometer.

Sample filtration

Open a bag of **LUMITUBE** and take one lumitube.

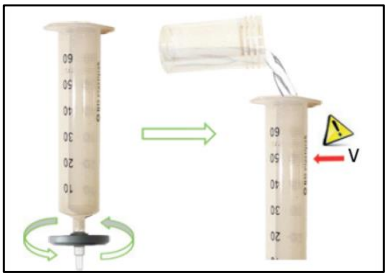
Remove the cap with the tube holder, and place the lumitube on the rack supplied.



Take the syringe out of its package.

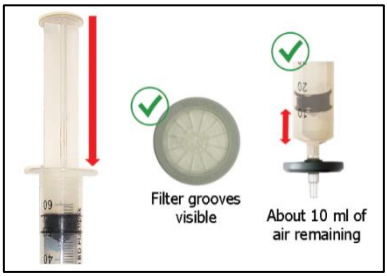
Remove the syringe plunger and put it down, being careful not to touch the lab bench with the black part.

Open the cap of the filter packaging (do not discard the plastic packaging).



Firmly screw the syringe on the filter to ensure it is watertight.
Pour the sampling container content into the syringe.

Write down the volume filtered.



Insert the plunger inside the syringe.

Filter all the sample until you feel a resistance. Then, stop pushing to avoid damage to the membrane.

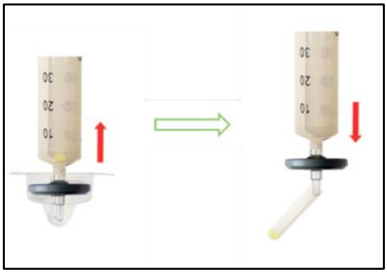
Do not press the plunger to the bottom of the syringe to avoid drying the filter.

Measurement

Make sure the reagent is close to room temperature (>18°C). Warm up the reagent in your hand if necessary.

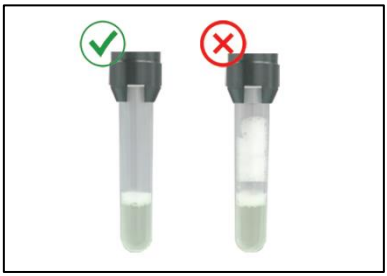
Put 4 drops of **EXTRACTANT** in the bottom of the plastic packaging of the filter.

From this step, no break time is allowed.



Place the filter tip in the bottom of the filter plastic packaging. Suck up all the **EXTRACTANT** through the filter and maintain the depression.

By a strong and constant pressure on the syringe plunger, push the liquid out of the syringe into the lumitube until a white foam comes out. To make it easier, push with the palm of your hand.



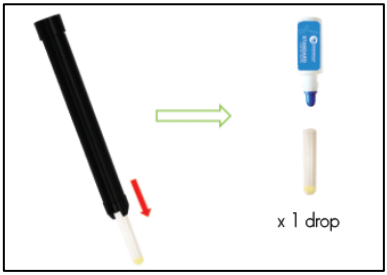
Stop the pressure as soon as the foam comes out. The picture opposite shows the aspect the foam must have in the lumitube.

A too strong or long pressure on the plunger will create a foam between the reagent and the top of the lumitube. This should be avoided to have a correct mixing of the **STANDARD** with the reagent.

Fix the lumitube to the tube holder.

Place them in the luminometer and press ENTER to start measurement.

Write down the R1 result (in RLU).



Immediately, get the tube holder with the lumitube out of the luminometer.

Take the lumitube off the tube holder and add one drop of **STANDARD** in the middle of the lumitube.

Fix the lumitube to the tube holder and **correctly homogenize the mix** by tapping the lumitube on a flat surface to get the foam down.

Place the tube holder with the lumitube in the luminometer and press ENTER.

Write down the R2 result (in RLU).

Interpretation of results

To interpret the results, you have two choices:

1. On smartphone or tablet: **LUMEN**

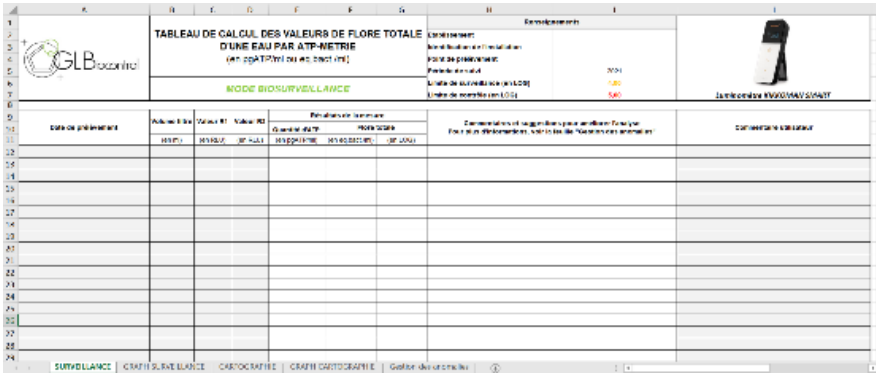
The app offers a combine analysis of ATP results and physicochemical values. It is a powerful **decision support tool**.

Enter the volume filtered, ATP values and the results of the physicochemical parameters measured. The app gives you an interpretation and saves your data. From the app, download the analytical report in pdf or the raw data in csv.

Load the app on your phone by scanning the QR Code!

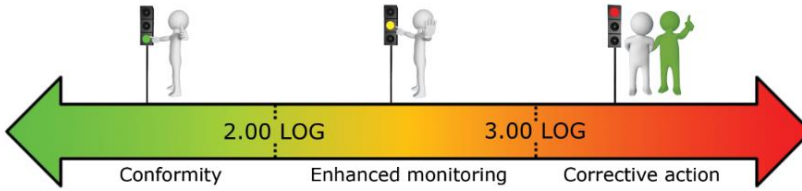
2. On computer: the Excel file

Enter the volume filtered, R1 and R2 values measured by the luminometer. Calculations are made automatically and a thanks to a color code, you are informed if a corrective action is required.

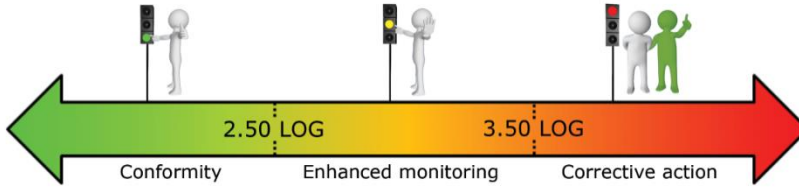


Warning and alarm thresholds were established based on our experience of water networks and on recommendations of the WHO. These thresholds should be refined based on the first results obtained on your network. The following arrows will help you **interpret the results**:

Drinking water production system (in LOG eq.bact./ml):



Drinking water supply network (in LOG eq.bact./ml):



We consider that when a measurement is:

- **Below the warning threshold**, the facility is under control,
- **Between the warning and the alarm threshold**, the facility does not present an immediate biohazard. A corrective action is recommended if 3 consecutive measurements are above the warning threshold,
- **Above the alarm threshold**, the facility is not under control. A quick corrective action is recommended.

In the Excel file, a color code informs you on the water quality.

Troubleshooting

Problem	Possible cause and correction
"Low sensitivity of the reagents. Increase the volume filtered" displayed in the Excel file.	The enzyme contained in the lumitube is not sufficiently active (out-of-date or degraded) or the **EXTRACTANT** is too cold. Warm up the reagent to a temperature above 18°C and filter a larger volume of water. If the problem remains, perform a ***Control of the reagent efficiency*** (cf. page 14).
"Control the mixing of the STANDARD, the temperature and condition of the reagents" displayed in the Excel file or "Measurement failed" on the WebApp.	Standardization was not successful. Tap the bottom of the lumitube on a flat surface, homogenize and restart the measurement. If the problem remains: - The enzyme contained in the lumitube is not sufficiently active (out-of-date or degraded) or the **EXTRACTANT** is too cold. Warm up the reagent and perform ***Control of the reagent efficiency*** (cf. page 14). - The sample has an inhibitory effect. Restart the analysis and rinse the membrane with sterile water or a specific solution after sample filtration (consult GL BIOCONTROL).
"Sample highly contaminated. If necessary, decrease the volume filtered" displayed in the Excel file.	The ATP concentration of the sample is too high. Restart the analysis with a smaller volume of sample (about one tenth).

Difficulty to obtain the foam.	Use the palm of the hand to push on the plunger and maintain the pressure few seconds. If the problem remains, use a 10 ml syringe or ask GL BIOCONTROL for advice.
Too much foam in the lumitube.	During manipulation, lean the lumitube so the reagent runs along the tube wall. Stop the pressure as soon as the foam comes out of the syringe. Properly homogenize the lumitube after **STANDARD** addition by tapping the bottom of the lumitube on a flat surface.
Low amount of reagent comes out of the syringe.	You probably have dried the filter. Restart the analysis making sure not to dry the filter during the filtration step. Stop pressure on the plunger when you feel a resistance.
Filter clogging.	It is possible to clog the filter if the sample is highly contaminated. - If you managed to filtrate at least 10% of the sample: write down the volume filtered, unscrew the filter and empty the syringe. Put the plunger back in the syringe placing the black Teflon part at 10 ml. Screw the filter back on the syringe and follow the classical protocol. - If you did not manage to filtrate the sample: pour only 5 ml of the sample in the syringe and complete to 50 ml with sterile water (consult GL BIOCONTROL).

Controls

To ensure proper functioning of your luminometer, we advise you to **conduct an annual maintenance**. GL Biocontrol offers this service.

Control of the luminometer contamination

- Place the tube holder in the luminometer and press ENTER,
- The result should be less or equal to 2 RLU. If not, with a cotton swab, wipe the internal surfaces of the measurement chamber.

Control of the reagent contamination

- In a lumitube, put 2 drops of **EXTRACTANT**,
- Fix the lumitube to the tube holder,
- Place it in the luminometer and press ENTER,
- The result should be less or equal to 5 RLU. If not, your **EXTRACTANT** dropper is probably contaminated. Contact GL Biocontrol for technical support.

Control of the reagent efficiency

- In a lumitube, put 2 drops of **EXTRACTANT** and 1 drop of **STANDARD** (reagent temperature must be above 18°C),
- Fix the lumitube to the tube holder,
- Properly homogenize the lumitube,
- Place it in the luminometer and press ENTER,
- For a good efficacy of the reagents, the result should be higher than 400 RLU. If not, the lumitubes are probably degraded. Discard the bag of **LUMITUBE** and choose a new one.

Contact

For further information or assistance on result interpretation, on the protocol or for commercial information, contact by email or by phone:

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A tutorial video is available on the USB flash drive supplied with the luminometer or on our website (https://www.gl-biocontrol.com/en/video-tutorial/), or here:

◀ **Video tutorial**

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