OPERATING MODE DENDRIDIAG® SW

Quantification of total flora by ATP-metry

- DRINKING WATER -

CONTENT

GL BIOCONTROL

GL BIOCONTROL specializes in environmental risk management and has an expertise 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: studies, 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.

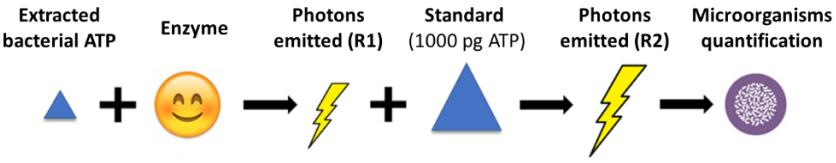
What is quantitative ATP-metry?

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. »

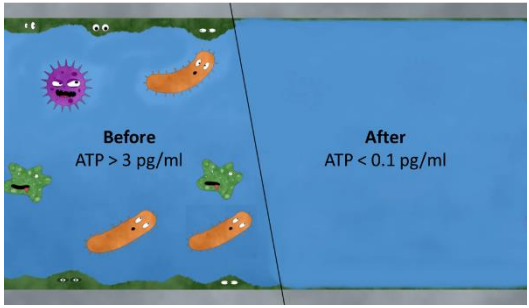
Once extracted from bacteria, the ATP is detected by bioluminescence, an easy enzymatic reaction which releases energy in the form of light. The amount of light emitted is proportional to the quantity of microorganisms. Each measurement is made quantitative because of the addition of a standard which takes into account environmental factors (temperature, pH, inhibitors…).



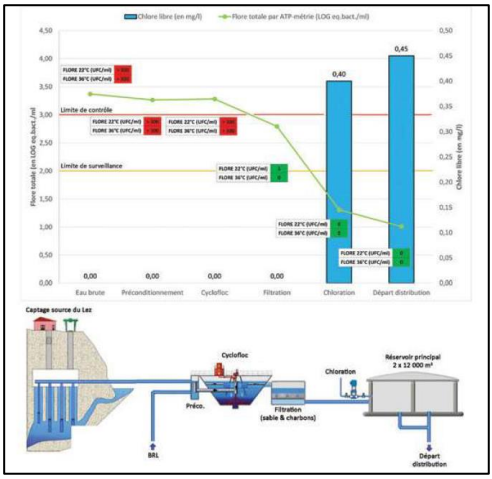
1 pg of ATP ≈ 1 000 bacteria

With this easy and reliable on-site analysis, you can validate treatments, look for critical points in your water network, or use it as a decision support tool.

Example of cleaning & disinfection

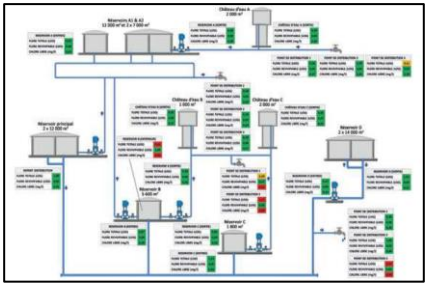


Applications



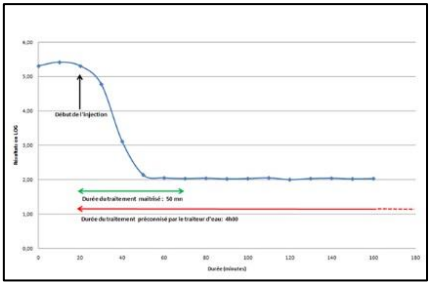
Monitoring of the drinking water treatment plants, from the source to the water conveyance:

- Mapping of a treatment plant and assessment of the treatment efficacy.
- On-site validation of the potabilization process.
- 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.



Assessment of the treatment efficiency:

- Return to service of a system (reservoir, 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.

Equipment needed

Kit of reagents for 60 measurements*

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

Kit of consumables for 60 measurements

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 (3 - 8°C).*

Protocol

Sampling

Specific protocol applies to sampling. The sampling outlet must be sterilized by a flame first. Let the water run to flush the sampling line until the piping capacity has been totally renewed (minimum 30 seconds for cold water and until you get a stable temperature for hot water. Place the plastic flask under the water flow without modifying the flow rate. Once filled, remove the plastic flask, close it and stop the flow.

Installation

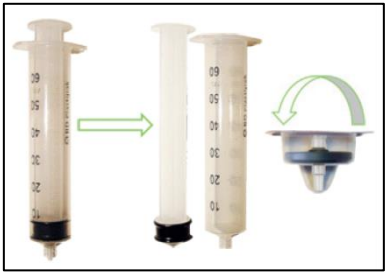
Take the dropper bottles of **EXTRACTANT** and **STANDARD** out as well as a **LUMITUBE** bag. Make sure they are at room temperature (above 18°C) before use.

Prepare the plastic consumables (sampling container, syringe and filter). Turn on the luminometer and wait 10 seconds for the device calibration.

Sample filtration

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

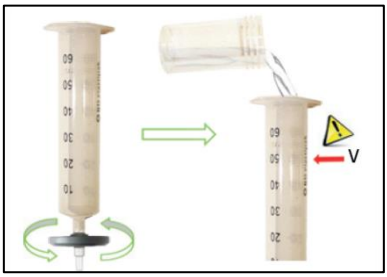
Remove the aluminium seal and place the lumitube on the rack supplied.



Take the syringe out of its package.

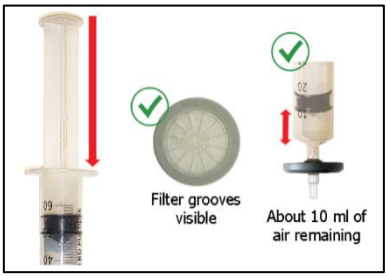
Remove the syringe piston and put it down, being careful not to touch the lab bench with the black part.

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 piston inside the syringe.

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

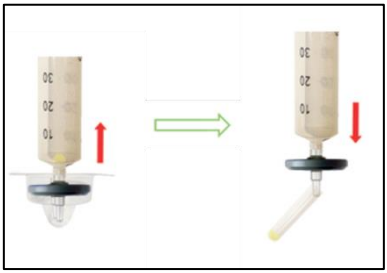
Do not press the piston to the bottom of the syringe to avoid drying the filter.

Measurement

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

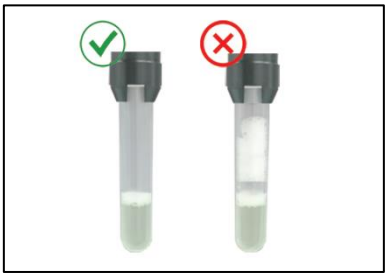
Put 4 drops of **EXTRACTANT** in the bottom of the plastic packaging of the filter.

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 piston, 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.



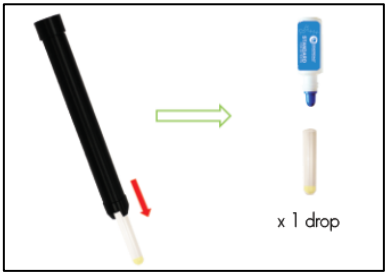
Stop the pressure as soon as the foam comes out. The picture opposite shows the aspect the foam must have in the lumitube.

A too strong or long pressure on the piston 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 the ENTER button 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 the ENTER button.

Write down the R2 result (in RLU).

Interpretation of results

Sampling date or location, volume filtered, R1 and R2 values measured by the luminometer must be entered in the table supplied.

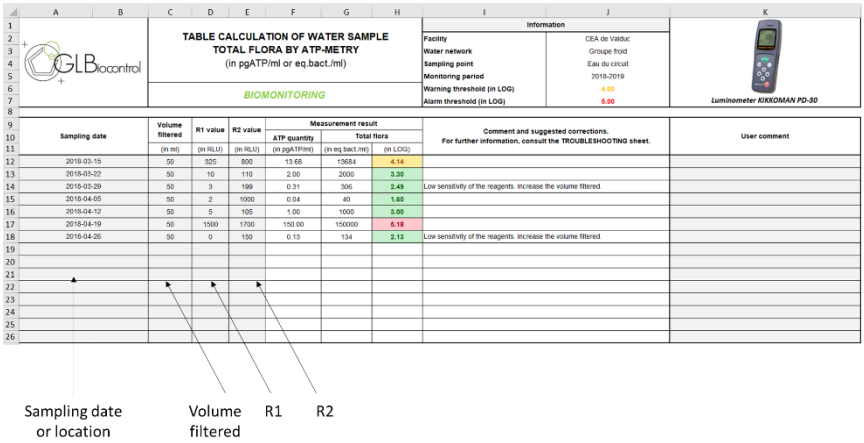


TABLE CALCULATION OF WATER SAMPLE
TOTAL FLORA BY ATP-METRY
(in pgATP/ml or eq.bact./ml)

BIOMONITORING

Information	
Facility	CEA de Valduc
Water network	Groupe froid
Sampling point	Eau du circuit
Monitoring period	2018-2019
Warning threshold (in LOG)	4.00
Alarm threshold (in LOG)	5.00

Luminometer KIKKOMAN PD-30

Sampling date	Volume filtered (in ml)	R1 value (in RLU)	R2 value (in RLU)	Measurement result: ATP quantity (in pgATP/ml)	Measurement result: Total flora (in eq.bact./ml)	Measurement result (in LOG)	Comment and suggested corrections. For further information, consult the TROUBLESHOOTING sheet.	User comment
2018-03-15	50	325	800	13.68	13684	4.14		
2018-03-22	50	10	110	2.00	2000	3.30		
2018-03-29	50	3	199	0.31	306	2.49	Low sensitivity of the reagents. Increase the volume filtered.	
2018-04-05	50	2	1000	0.04	40	1.60		
2018-04-12	50	5	105	1.00	1000	3.00		
2018-04-19	50	1500	1700	150.00	150000	5.18		
2018-04-26	50	0	150	0.13	134	2.13	Low sensitivity of the reagents. Increase the volume filtered.	

Sampling date or location — Volume filtered — R1 — R2

The table automatically performs the calculation. Results are given in:

- **picogram** per milliliter (pg ATP/ml),
- total flora in **equivalent bacteria** per milliliter (eq.bact./ml),
- total flora in **logarithm** per milliliter (LOG eq.bact./ml).

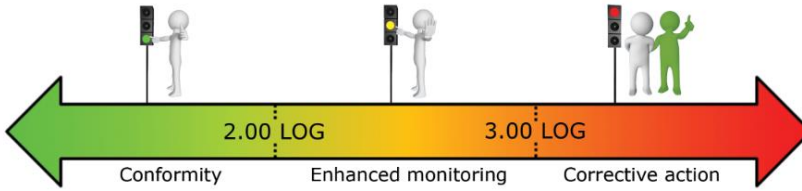
In case you **monitor your water network over time**, fill in the « BIOMONITORING » sheet.
A graph is automatically drawn in the « GRAPH BIOMONITORING » sheet.

In case you **do a cartography of your water network**, fill in the « CARTOGRAPHY » sheet.
A graph is automatically drawn in the « GRAPH CARTOGRAPHY » sheet.

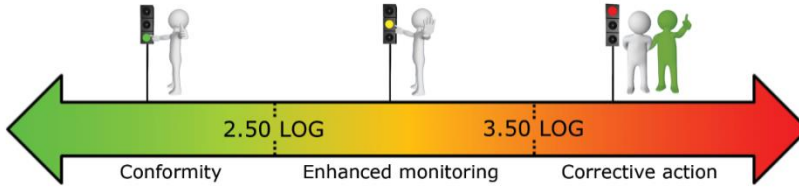
Two results are considered significantly different if the difference is superior to 1.00 LOG.

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 piston 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 piston 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 piston 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. We remain at your disposal to assist you.

Control of the luminometer contamination

- Place the tube holder in the luminometer,
- Press the ENTER button,
- 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 the ENTER button,
- The result should be less or equal to 5 RLU. If not, your dropper bottle of **EXTRACTANT** 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 the ENTER button,
- For a good efficacy of the reagents, the result should be higher than 400 RLU. If not, the lumitube are probably degraded. Discard the bag of **LUMITUBE** and choose a new one.

Contact

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

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A tutorial video of the protocol is available on the flash drive supplied with the luminometer or on our website:

https://gl-biocontrol.com/video-tutorials

In addition of the Excel file, the web application **DENDRIDIAG® APP** is available on smartphone and tablet at:

https://dendridiag.gl-biocontrol.com

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