OPERATING MODE DENDRIDIAG® BF

Quantification of total flora on surfaces by ATP-metry

- SURFACES (FOOD INDUSTRY & PIPINGS…) -

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.

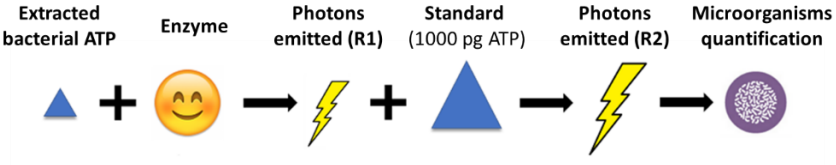
What is quantitative ATP-metry?

ATP-metry is a microbiological technique that evaluates the **overall bacterial load** of a surface 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…).

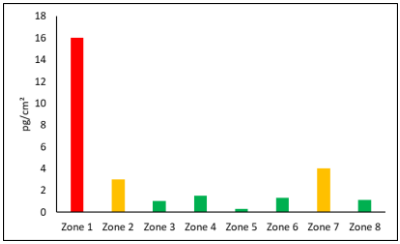


1 pg of ATP ≈ 1 000 bacteria

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.

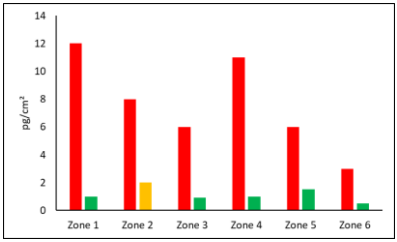
Applications

With GL BIOCONTROL's ATP-metry, you can:



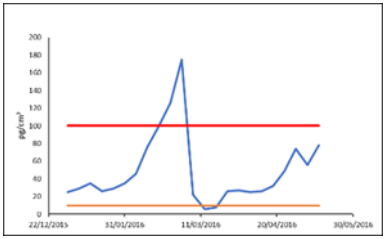
Control the facility cleanliness:

- Manage biofouling.
- Improve health risk management.
- Avoid production shutdown.



Assess operating procedure efficiency:

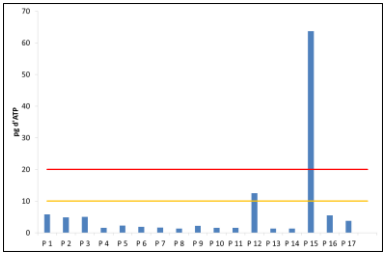
- Validate efficiency of:
  - Cleaning,
  - Disinfection.
- Validate treatment strategy.



Manage biofilm formation in a pipe:

- Control and assess the fouling state of the pipe network.

Start and validate cleaning and disinfection procedures.



Identify the critical areas:

- Determine critical areas with important biomass growth.
- Adapt cleaning and disinfection strategy.
- Highlight malfunctions.

Equipment needed

Kit of reagents for 50 measurements*

Product	Quantity
Dropper bottle **EXTRACTANT**	2
Dropper bottle **STANDARD**	1
Aluminium bag of 10 test tubes **LUMITUBE**	5

Kit of consumables for 50 measurements

Product	Quantity
Sterile swabs	50

Equipment

Product	Quantity
Stainless steel sampling template	1
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

Installation

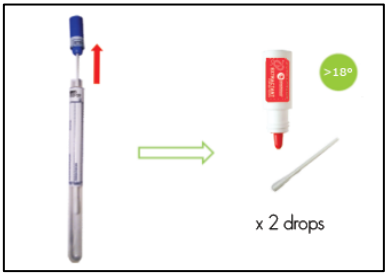
Take the dropper bottles of **EXTRACTANT** and **STANDARD** out as well as a **LUMITUBE** pouch. Make sure they are at room temperature (above 18°C) before use.

Take out the swabs as well as the sampling template. Turn on the luminometer and wait 8 seconds for the device calibration.

Sampling

Take the required number of lumitubes out of the aluminium bag and place them on the rack supplied.

Write the sampling point on each swab.



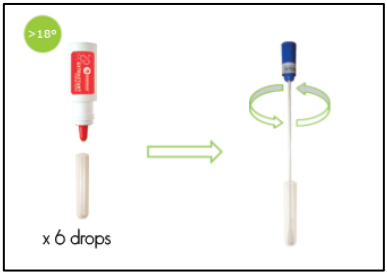
Get the swab out of its packaging being careful not to touch the cotton tip with your fingers or with the lab bench.

Squeeze 2 drops of **EXTRACTANT** on the tip and put the swab back in its packaging.

Clean the sampling template and place it on the surface.

Thoroughly swab the 20cm² surface. Pass at least two times on the surface on two different directions.

Measurement



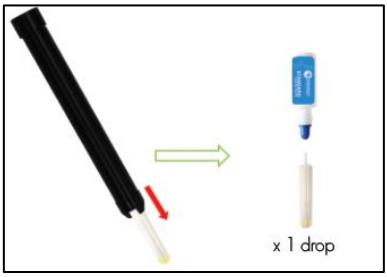
Squeeze 6 drops of **EXTRACTANT** in the lumitube.

Insert the swab in the lumitube and homogenize correctly by turning the swab using the rod.

Break off the top of the swab while maintaining it in the lumitube.

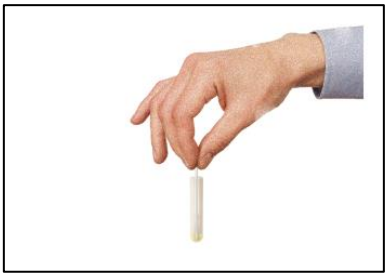
Fix the tube to its holder. Place them in the luminometer and press ENTER to start the measurement.

Write down the R1 result (in RLU).



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

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



Correctly homogenize the mix by turning the swab inside the test tube to get a good calibration.

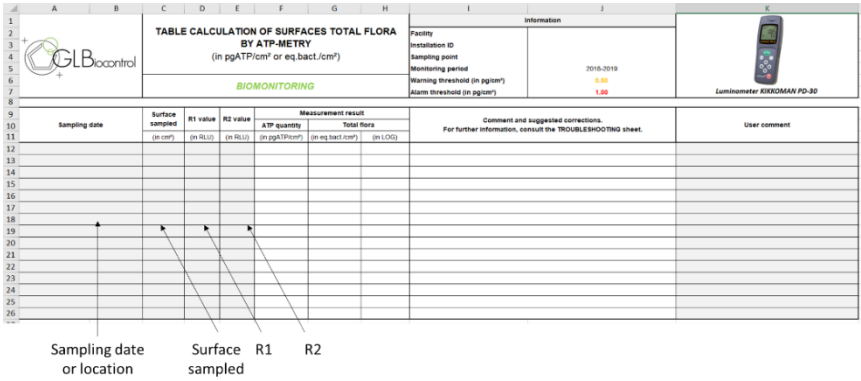
Fix the tube to its holder.

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

Write down the R2 result (in RLU).

Interpretation of results

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



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

- **picogram** per square centimeter (pg ATP/cm²),
- total flora in **equivalent bacteria** per square centimeter (eq.bact./cm²),
- total flora in **logarithm** per square centimeter (LOG eq.bact./cm²).

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

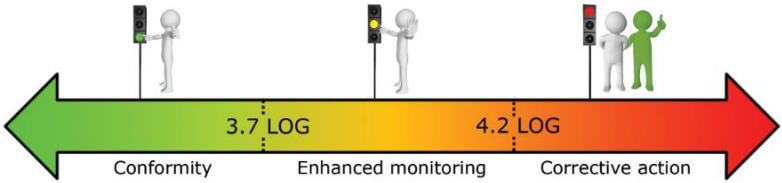
⇨ *It is advisable to monitor, at least once a week, the critical areas.*

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

⇨ *It is advisable to perform a measurement before and after cleaning or disinfection procedure of each critical area.*

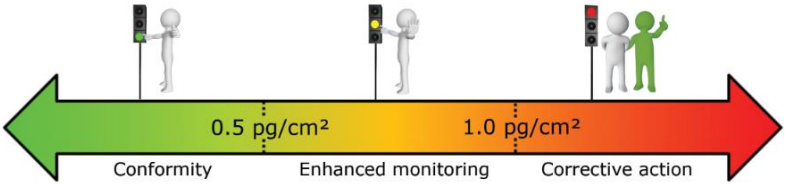
Warning and alarm thresholds were established based on our experience. These thresholds should be refined based on the first results obtained on your **facility**. The following arrows will help you **interpret the results**:

Operating swimming pools (in LOG eq.bact./cm²):

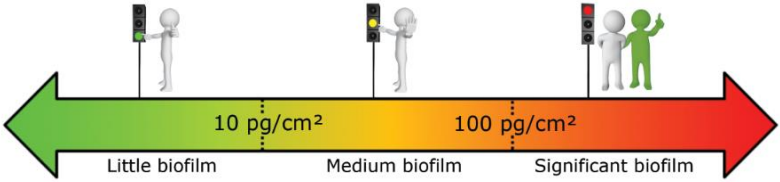


NB: a handbook is specially dedicated to this application.

Surfaces of food processing industry (in pgATP/cm²):



Surfaces of water pipes (in pgATP/cm²):



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, the result is displayed in green, orange or red depending if it is under the warning threshold, between the warning and the alarm threshold or above the alarm threshold.

Troubleshooting

Problem	Possible cause and correction
"Low sensitivity. If necessary, sample a larger surface." 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 1000, the temperature and condition of the reagents." displayed in the Excel file.	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).
"Sample highly contaminated. If necessary, sample a smaller surface." displayed in the Excel file.	The ATP concentration of the sample is too high. Restart the analysis by sampling a smaller surface.

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 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 4 drops of **EXTRACTANT**,
- Fix the lumitube to its holder,
- Place it in the luminometer and press ENTER,
- 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 its 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 lumitube are probably degraded. Discard the **LUMITUBE** pouch 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 of the protocol is available on the flash drive supplied with the luminometer or on our website:

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

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