OPERATING MODE DENDRIDIAG® BF

Quantification of total flora on surfaces by ATP-metry

- LUMINOMETER KIKKOMAN PD30 -

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:

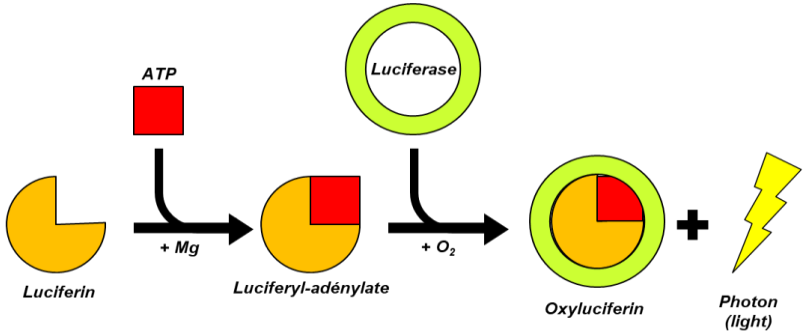
- **Develops** risk management tools (ATP-metry kits for total flora quantification, DNA extraction purification kits, real time PCR amplification kits…).
- **Uses** methodologies and innovative tools to study the microbial world (qPCR, NGS, ATP-metry…).
- **Studies** ecosystems to anticipate and prevent 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.

What is ATP-metry?

Adenosine triphosphate (ATP) is the major intermediary energy required in most cellular metabolism reactions. Every living cell produces and consumes ATP. This coenzyme, specific to living environments, proves the existence of living organisms.

Quantifying ATP equates to **quantifying total microorganisms** (or total biomass).

To perform this type of assay, the light emitted by the enzymatic reaction of **bioluminescence** using luciferin and firefly luciferase is measured (see below).



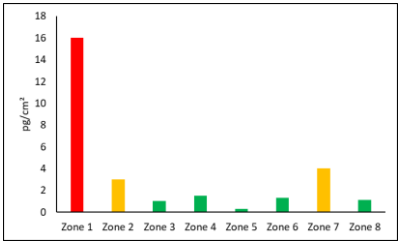
ATP, in the presence of a luciferin/luciferase complex with a catalyst, releases energy in the form of light. By measuring the amount of light emitted using a **luminometer**, we deduce the **quantity of ATP in picogram per cm²**. The total flora, expressed in equivalent bacteria per cm², is calculated from the following:

1 picogram ≈ 1 000 bacteria.

The ATP-metry measurement method is a **field test** whose result is obtained in few minutes.

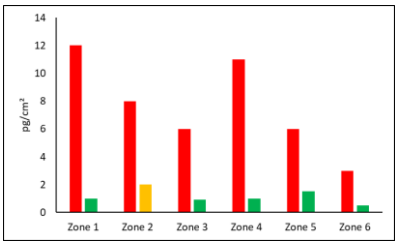
GL BIOCONTROL's ATP-metry

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



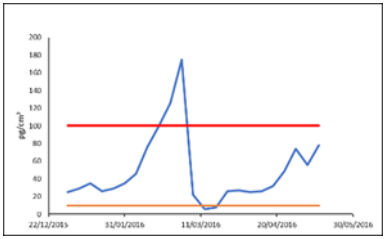
Control the facility cleanliness:

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



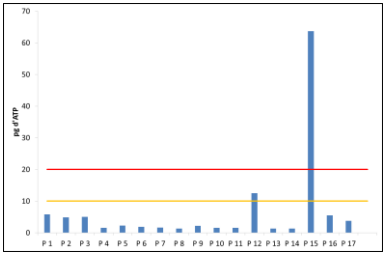
Assess operating procedure efficiency:

- Validate efficiency of:
  - Cleaning (bio-dispersant),
  - Disinfection (biocide).
- 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 100 measurements

Product	Quantity
Dropper bottle **DENDRIDIAG® BF ***	5
Dropper bottle **EXTRACTANT** *	5
Dropper bottle **STANDARD 1000 ***	2

Kit of consumables for 100 measurements

Product	Quantity
Sterile swabs	100
Disposable sterile polypropylene test tubes	100

Equipment

Product	Quantity
Stainless steel sampling template	1
Luminometer KIKKOMAN PD30 or equivalent	1
Tube holder for PD30	1

** Reagents (**DENDRIDIAG® BF**, **EXTRACTANT** and **STANDARD 1000**) should be stored in the dark in a freezer (-18°C). In this way, they can be kept for at least 12 months. After first use, the reagents should preferentially be refrozen. Or else, they can be kept refrigerated (between 3 and 8°C) for a maximum of 8 consecutive weeks.*

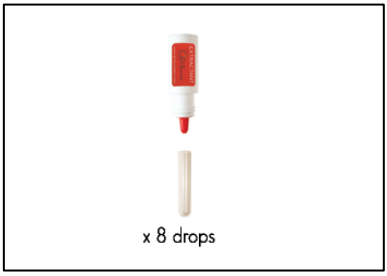
Protocol

Installation

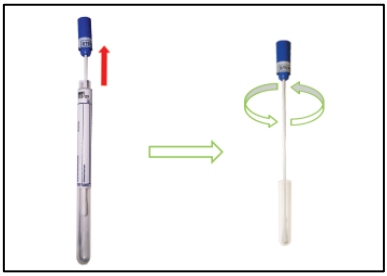
Thaw a dropper bottle of each reagent (**DENDRIDIAG® BF**, **EXTRACTANT** and **STANDARD 1000**). Bring them to room temperature (above 18°C).

Prepare the plastic consumables (swab and test tube) and the sampling template. Turn on the luminometer and wait 10 seconds for the device calibration.

Sampling



Make sure the reagent **EXTRACTANT** is close to room temperature (> 18°C) and put 8 drops of **EXTRACTANT** in the middle of the test tube.



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

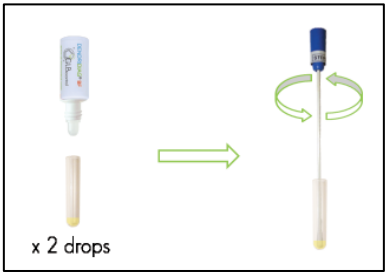
Plunge the cotton tip in the test tube containing the **EXTRACTANT**.

Place the sampling template on the surface you want to control.

Scrub the surface (20cm²) with the swab. Pass at least two times on the surface on two different directions.

From this step, no break time is allowed.

Measurement



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

Add 2 drops of DENDRIDIAG® BF in the middle of the test tube containing the **EXTRACTANT.**

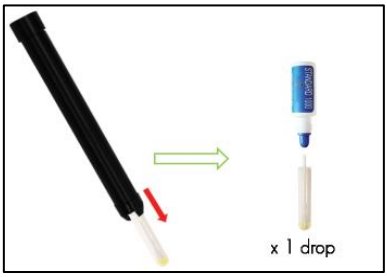
Put the swab in the test tube and homogenize the mix by turning he swab.

Break the swab while maintaining it in the test tube.

Fix the tube to the tube holder.

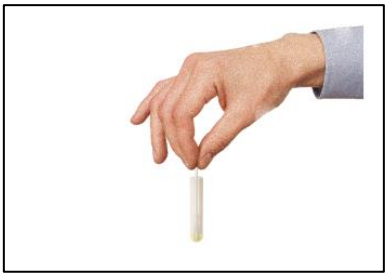
Place them in the luminometer and press the ENTER button to start measurement.

Write down the R1 result (in RLU).



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

Take the test tube off the tube holder and add one drop of **STANDARD 1000** in the middle of the test tube.



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

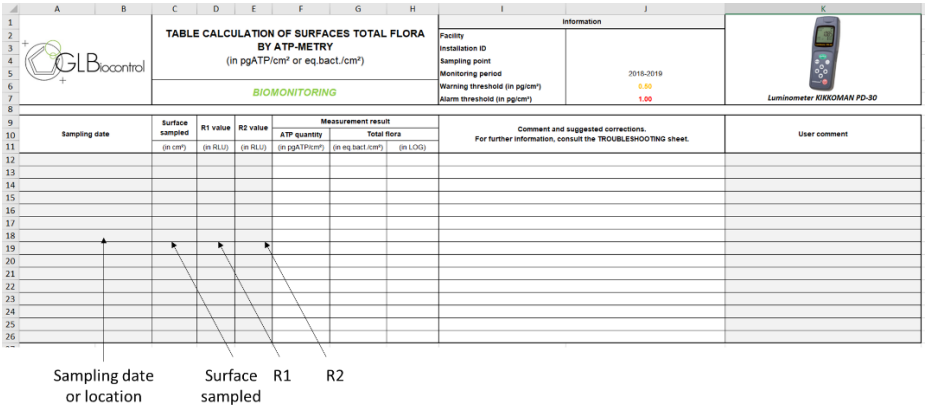
Fix the tube to the tube holder.

Place them in the luminometer and press the ENTER button 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.

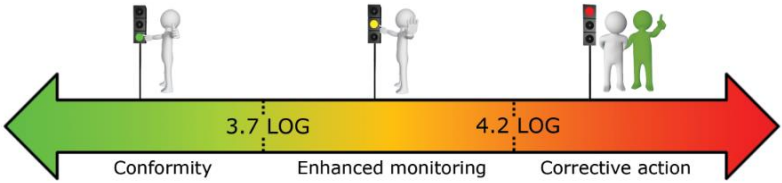
⇨ *It is advisable to monitor, at least once a week, the critical areas.*

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.*

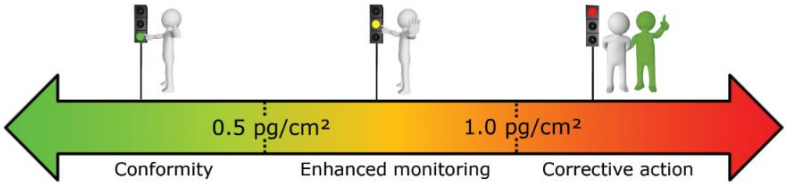
Warning and alarm thresholds were established based on our experience. These thresholds should be refined based on the first results obtained on your **facility**. The following arrows will help you **interpret the results**:

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

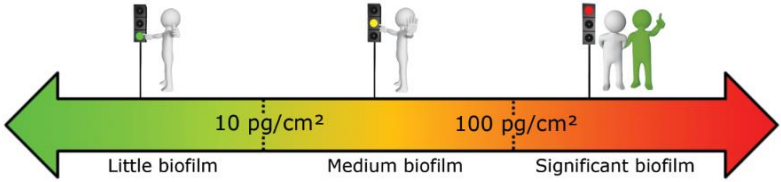


NB: a handbook is specially dedicated to this application.

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 reagent **DENDRIDIAG® BF** is not sufficiently active (out-of-date, degraded or too cold) to obtain high sensitivity. Warm up the reagents **DENDRIDIAG® BF** and **EXTRANCTANT** and sample a larger surface if necessary. 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 test tube on a flat surface and homogenize the mix by turning the swab in the test tube. Restart the measurement. If the problem remains, the reagent **DENDRIDIAG® BF** is not sufficiently active (out-of-date, degraded or too cold). Warm up the reagents **DENDRIDIAG® BF** and **EXTRANCTANT**, and perform a ***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

Control of the luminometer contamination

- Fix an empty test tube to the tube holder,
- Place them in the luminometer and 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 test tube, put 2 drops of **DENDRIDIAG® BF** and 4 drops of **EXTRACTANT**,
- Fix the test tube to the tube holder,
- Place them in the luminometer and press the ENTER button,
- The result should be less or equal to 5 RLU. If not, discard the contaminated reagents and select a new bottle of **DENDRIDIAG® BF** and of **EXTRACTANT.**

Control of the reagent efficiency

- In a test tube, put 2 drops of **DENDRIDIAG® BF**, 4 drops of **EXTRACTANT**, and 1 drop of **STANDARD 1000** (reagent temperature must be above 18°C),
- Fix test tube to the tube holder,
- Properly homogenize the tube by tapping the bottom of the tube on a surface,
- Place them in the luminometer and press the ENTER button,
- For a good efficacy of the reagents, the result should be higher than 130 RLU. If not, discard the reagents and select a new bottle of **DENDRIDIAG® BF** and of **EXTRACTANT.**

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 USB key supplied with the luminometer or on our website in the tab Products – ATPmetry kit for surfaces:

www.gl-biocontrol.com

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