CLEANING AND DISINFECTION MANAGEMENT OF SWIMMING POOL SURFACES

QUANTITATIVE ATP-METRY DENDRIDIAG BF

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Use of a cleanliness indicator

If water quality has always been a major concern of the Health Regional Agencies and authorities, hygiene conditions in swimming pool facilities are also characterized by cleanliness of the changing rooms, sanitary facilities (toilets, showers, foot bathes) and of pool decks. Indeed, insufficient maintenance of floor cleanliness can lead to various skin conditions for the customers (verruca, fungal infections…). Furthermore, it contributes to give a bad impression of the swimming pool, which strongly influences visits.

Unlike microbial water quality, until now, floor cleanliness was mainly assessed by visual inspection rather than specific analyses. After several months of testing carried out in collaboration with the French Health Regional Agencies, two complementary indicators were chosen:

- total count of microorganisms per 20 cm^2,
- quantification of organic residues present on the floor by quantitative ATP-metry.

This work compared the practices of different facilities and gave more reliable assessment criteria than simple visual inspection to swimming pool managers. Warning and alarm thresholds were established in order to implement suitable maintenance and cleaning protocols and to assess quality of work.

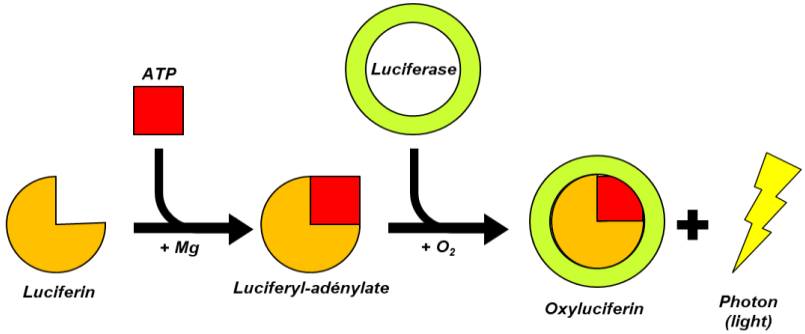
This handbook describes how to use quantitative ATP-metry in order to monitor and control surface cleanliness of swimming pools.

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).



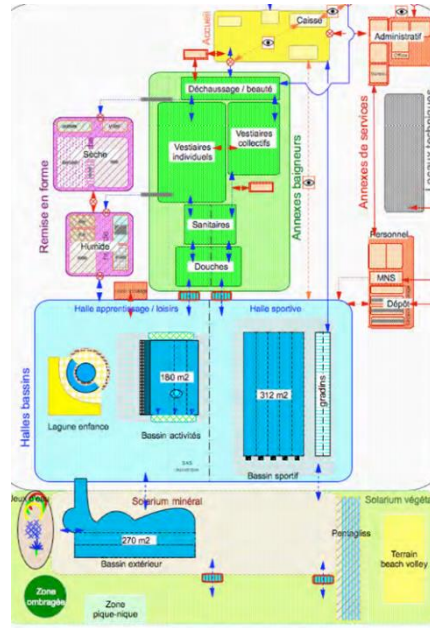
ATP, in the presence of a luciferin/luciferase complex with a catalyst, releases energy in the form of light. By measuring the amount of light emitted using a **luminometer**, we deduce the **quantity of ATP in picogram per cm²**. The total flora, expressed in equivalent bacteria per cm², is calculated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

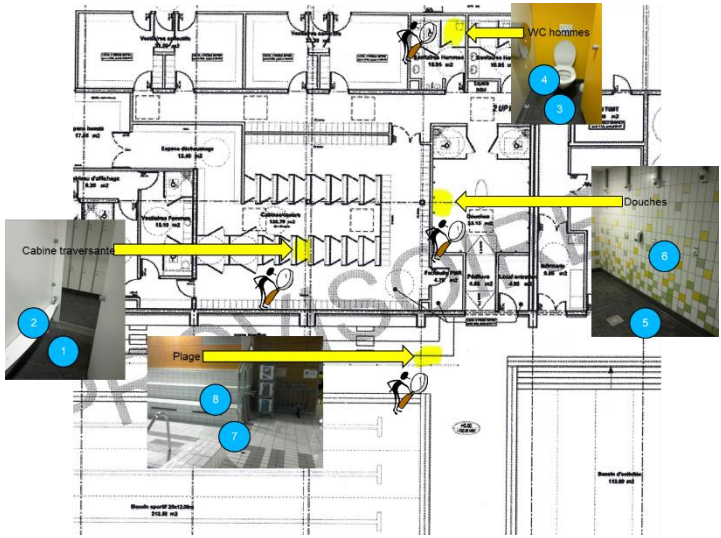
1 picogram ≈ 1 000 bacteria.

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

How to correctly manage cleanliness of swimming pool surfaces?

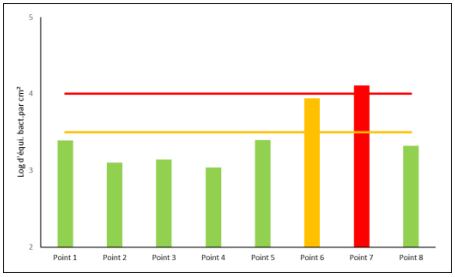
- **Determine** the critical areas,
- **Define** a cleaning frequency appropriate with the functioning facility constraints (morning, evening…),
- **Identify** the nature of the products used (active ingredient) and their operating modes (dilution…),
- **Select** the appropriate material for the diverse interventions,
- **Establish** substantial and adequate human resources,
- **Involve** the cleaning staff.





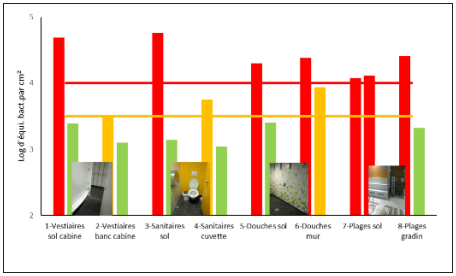
Why use ATP-metry to monitor swimming pools?

With quantitative ATP-metry, you can:



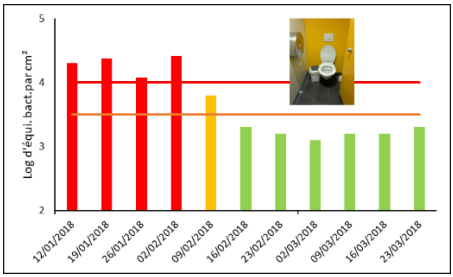
Control the facility cleanliness before opening:

- Manage biofouling.
- Improve health risk management.



Assess operating procedure efficiency:

- Validate efficiency of:
  - Cleaning (bio-dispersant),
  - Disinfection (biocide).
- Validate treatment strategy.



Identify the critical areas:

- Determine critical areas with important biomass growth.
- Adapt cleaning and disinfection strategy.
- Monitor development.

Work performed in collaboration with the French Health Regional Agencies (ARS).

Quantitative ATP-metry also exists for sanitary water monitoring with the **DENDRIDIAG®** **SW** kit. Assessment of the microbial water quality of the pools and monitoring of the different steps of water treatments are essential for a good management of swimming pool facilities.

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.*

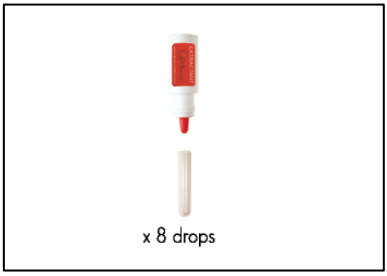
Protocol

Installation

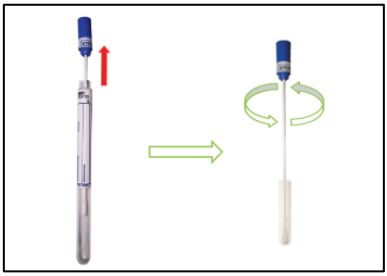
Thaw a dropper bottle of each reagent (**DENDRIDIAG® BF**, **EXTRACTANT** and **STANDARD 1000**). Bring them to room temperature (above 18°C).

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.

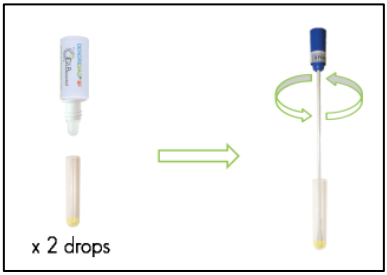
Plunge the cotton tip in the test tube containing the **EXTRACTANT**.

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.**

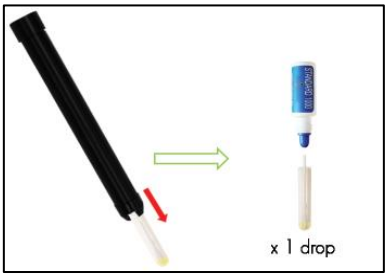
Put the swab in the test tube and homogenize the mix by turning he swab.

Break the swab while maintaining it in the test tube.

Fix the tube to the tube holder.

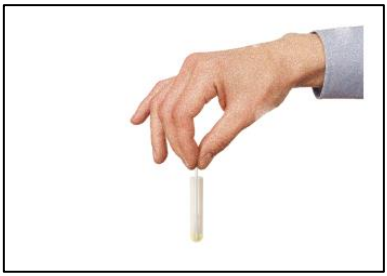
Place them in the luminometer and press the ENTER button to start measurement.

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.

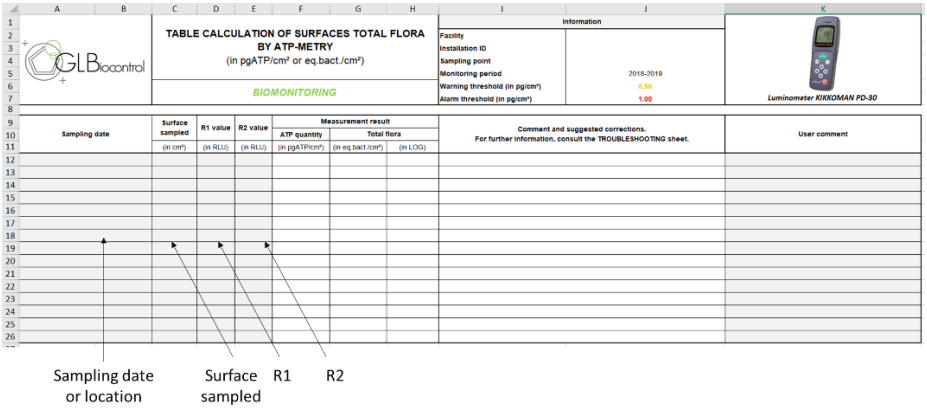
Fix the tube to the tube holder.

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.

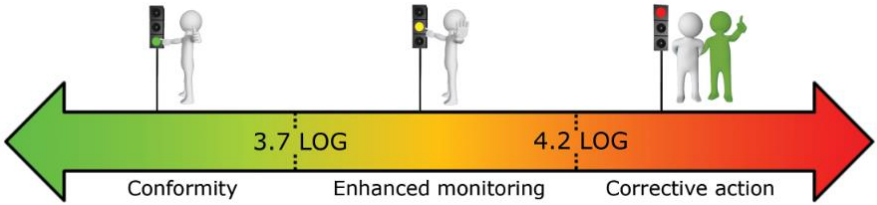
⇨ *It is advisable to monitor, at least once a week, the critical areas. As a minimum, they are 8 areas to analyze: the floor and benches of the changing rooms, floor and toilet bowl of the sanitary facilities, floor and walls of the showers, floor and benches of the pool deck area. For spas, it is important to monitor floor and bench seats.*

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

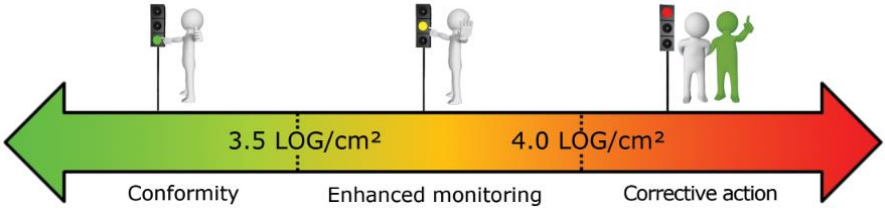
⇨ *It is advisable to perform a measurement before and after cleaning or disinfection procedure of each critical area.*

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):



Operating swimming pools (in LOG eq.bact./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,
- **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 15).
"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 15).
"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:

Yannick FOURNIER
Sales engineer
9, avenue de l'Europe - Cap Alpha
34 830 CLAPIERS (FRANCE)
Phone: +33 (0)6 33 64 42 29
Email: y.fournier@gl-biocontrol.com

Clement FAYE
Research engineer
9, avenue de l'Europe - Cap Alpha
34 830 CLAPIERS (FRANCE)
Phone: +33 (0)6 72 70 46 98
Email: c.faye@gl-biocontrol.com

A tutorial video of the protocol is available on the USB key supplied with the luminometer or on our website in the tab Products – ATP-metry kit for surfaces:

www.gl-biocontrol.com

9, avenue de l'Europe - Cap Alpha
34 830 CLAPIERS (FRANCE)
Phone: +33 (0)9 67 39 35 20
Email: contact@gl-biocontrol.com
Web: www.gl-biocontrol.com