GLBiocontrol
PROCEDURES

DENDRIDIAG® BF

OPERATING MODE FOR QUANTIFICATION OF TOTAL FLORA ON SURFACES BY ATP-METRIE

- LUMINOMÈTER KIKKOMAN PD30 -

VERSION: V2018-10

GL BIOCONTROL – Company specialized in bioenvironmental risk management of water
9, avenue de l'Europe - CAP ALPHA - 34 830 CLAPIERS - FRANCE / Phone : +33.(0)9.67.39.35.20 - Fax : +33.(0)9.55.25.40.31
Email: contact@gl-biocontrol.com - Web: www.gl-biocontrol.com

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GL BIOCONTROL overview

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, electropositive membranes for viruses…).
- **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.

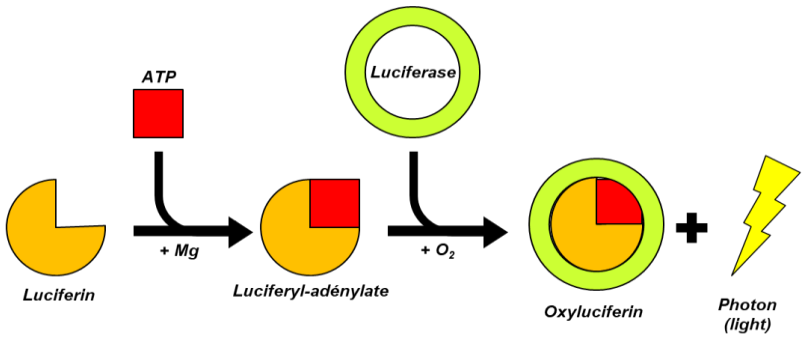
GL BIOCONTROL offers everything you need to quantify total flora in waters, on surfaces and in the air by **ATP-metry**: measurement kits **DENDRIDIAG®**.

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 flora).

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 square centimeter**. The total flora, expressed in equivalent bacteria per square centimeter, 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.

Why use ATP-metry for microbiological monitoring?

Contamination or degradation of surface microbial quality is caused by one or all of the following: dubious cleaning and disinfection procedures, nature of the biocide used, low quality of the surface material. There is no definitive solution to eradicate microbiological issues. Only an approach starting with the **observation** of the surfaces state, followed by the **monitoring** of the corrective action effects can result in effective management of microbiological risks.

Most of the regulatory texts relating to microbiological risk management of surfaces, require that the facility manager uses **indicators** to follow and anticipate a microbiological shift of their facilities, in order to avoid contaminants such as (*Campylobacter*, *Pseudomonas* or *Salmonella* for example).

The monitoring indicator should be a technology that is **rapid**, **reliable**, **easy to use** and **economic** in order to frequently analyze the facility.

Among the different microbiological indicators, the most frequent are heterotrophic plate count at 22°C or 36°C, quantitative PCR, qualitative ATP-metry and **quantitative ATP-metry**.

Quantitative ATP-metry is one of the best indicators for biological monitoring. Using quantitative ATP-metry, you will:

- **Control the facility cleanliness:** manage biofouling, improve health risk management, Avoid production shutdown due to non-compliance with the regulatory requirement (*Pseudomonas, Campylobacter*…).
- **Assess operating procedure efficiency:** validate efficiency of cleaning (bio-dispersant) and disinfection (biocide) procedures, 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 strong 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	

*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 will be preferentially refrozen. Or else, they can be kept refrigerated (between 3 and 8°C) for a maximum of 8 consecutive weeks.*

Kit of consumables for 100 measurements

Product	Quantity	
Sterile swabs	100	
Disposable sterile polypropylene test tubes	100	

Equipment

Product	Quantity	
Stainless steel sampling template (20 cm²)	1	
Luminometer KIKKOMAN PD30 or equivalent	1	
Tube holder for PD30	1	
Fridge (3 to 8°C) *	1	
Freezer (≈ -18°C) *	1	

** For good conservation of the reagents, it is necessary to store them in a freezer or at least in a fridge.*

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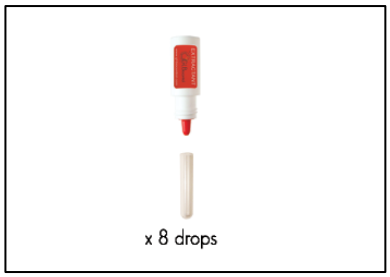
Protocol for quantification of total flora on surfaces

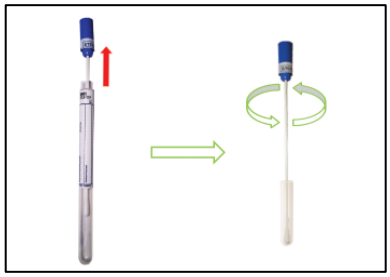
Phase 1: installation

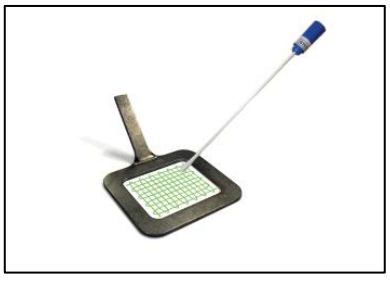
1. Thaw a dropper bottle of each reagent: DENDRIDIAG® **BF**, **EXTRACTANT** and **STANDARD 1000**. Bring them to room temperature (above 18°C),
2. Prepare the plastic consumables (swab and test tube) and the sampling template,
3. Turn on the luminometer and wait 10 seconds for the device calibration.

*To perform the analysis, the reagents DENDRIDIAG® **BF**, **EXTRACTANT** and **STANDARD 1000** must be at room temperature (between 18°C and 25°C) to ensure a maximal enzyme efficiency.*

Phase 2: sampling







1. 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,
2. Get the swab out of its packaging being careful not to touch the cotton tip with your fingers or with the lab bench,
3. Plunge the cotton tip in the test tube containing the **EXTRACTANT**, turn the swab in the test tube to humidify it,
4. Place the sampling template on the surface you want to control,
5. Scrub the surface defined by the sampling template (20cm²) with the swab. Pass at least two times on the surface on two different directions,

From this step, no break time is allowed.

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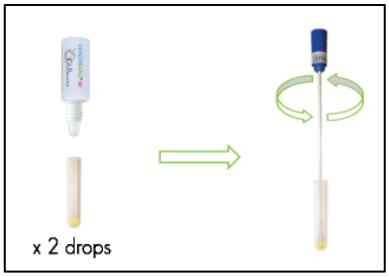
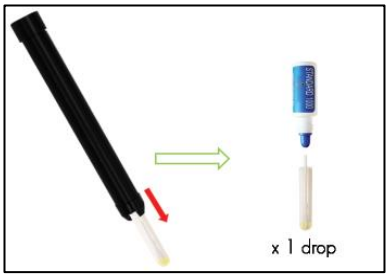
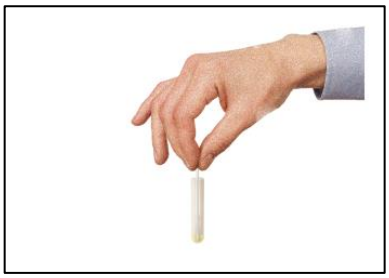
Phase 3: measurement

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

6. Add 2 drops of DENDRIDIAG® BF in the middle of the test tube containing the **EXTRACTANT**,
7. Put the swab in the test tube and homogenize the mix by turning the swab,
8. Break the swab while maintaining it in the test tube. Do not touch the cotton tip of the swab with your fingers,
9. Fix the tube to the tube holder,
10. Place them in the luminometer and press the ENTER button to start measurement,
11. After 10 seconds of measurement, write down the R1 result in RLU (Relative Light Unit),
12. Immediately, get the tube holder with the test tube out of the luminometer,
13. Take the test tube off the tube holder and add one drop of **STANDARD 1000** in the middle of the test tube. The dropper bottle must not touch the tube while adding the drop,
14. Correctly homogenize the mix by turning the swab inside the test tube to get a good standardization,
15. Fix the tube to the tube holder,
16. Place them in the luminometer and press the ENTER button to start measurement,
17. After 10 seconds of measurement, write down the R2 result in RLU (Relative Light Unit).







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Result analysis

Calculation of total flora quantity

The intracellular ATP concentration is expressed in picogram ATP per square centimeter. To obtain the result, execute the following operations:

Calculation of the standard (in RLU/pgATP):

$$\text{STANDARD} = \frac{\text{R2 - R1}}{\text{1 000}}$$

Calculation of the biomass value (in pgATP/cm²):

$$[\text{ATP}] = \frac{\text{R1}}{\text{STANDARD x S}}$$

With:

R1 = result obtained on the sample in RLU,

R2 = result obtained on the sample + **STANDARD 1000** in RLU,

STANDARD = value of the **STANDARD 1000** in RLU/pgATP,

S = surface sampled (defined at 20 cm² with the sampling template).

It is possible to convert the ATP concentration (in pgATP/cm²) to equivalent bacteria per square centimeter (eq. bact./cm²) using the following rule: 1 picogramme ATP ≈ 1 000 bacteria.
For example, 5 pg/cm² ≈ 5000 eq.bact./cm².

Data management with Excel

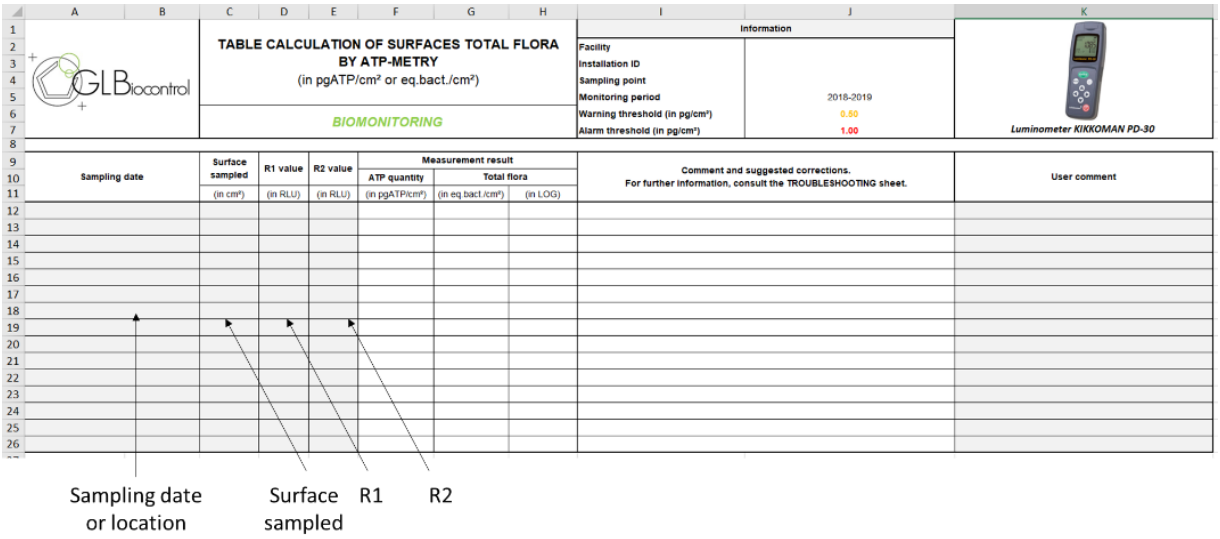
An Excel file is supplied by GL BIOCONTROL to automatically perform the calculations presented above. The file must be used to monitor your facility and interpret the values obtained.

After each analysis, sampling date or location, surface sampled, R1 and R2 values measured by the luminometer must be entered in the table supplied. Only the grey columns must be completed.

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).

By default, we use the result in logarithm equivalent bacteria per square centimeter. However, you can use one of the three units.



In case you **monitor your facility over time**, fill in the « BIOMONITORING » sheet. Each line corresponds to one day of measurement. 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. Each line corresponds to a point of sampling. A graph is automatically drawn in the « GRAPH CARTOGRAPHY » sheet. If you want to perform several cartographies, you have to duplicate the sheet or replace the previous values.

⇨ *It is advisable to perform a measurement before and after each cleaning and disinfection of the critical areas.*

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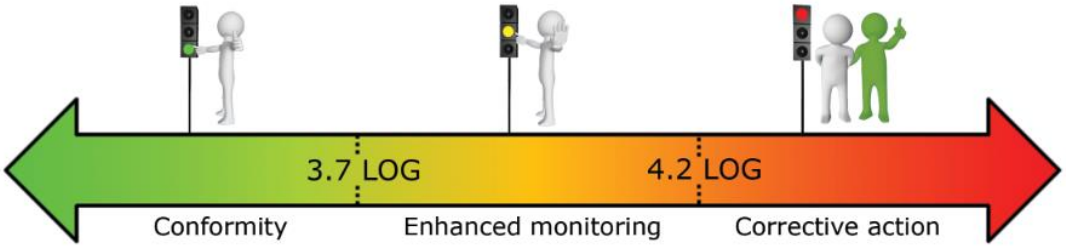
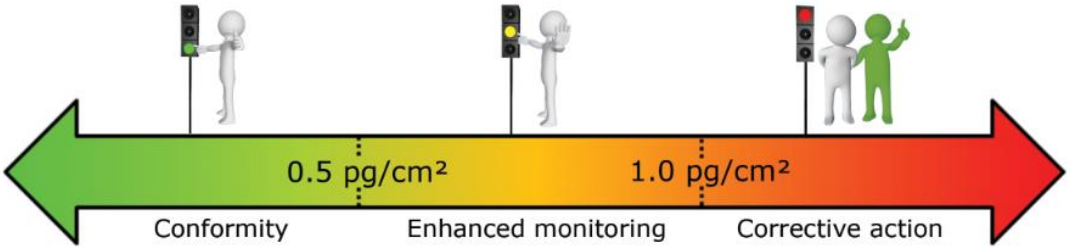
Result interpretation

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

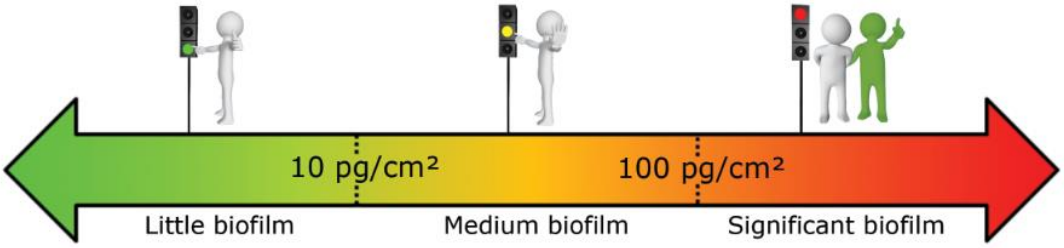
Operating swimming pools (in LOG/cm²):



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 surface is under control,
- **Between the warning and the alarm threshold**, the surface 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 surface 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.
In order to help understanding the results, we encourage you to write down any relevant information in the “user’s comment” column.

Troubleshooting

The Excel file automatically detects 3 types of error and displays a message explaining the issue identified. When an error is highlighted, please refer to the troubleshooting table below.

If a measurement must be repeated after an error, we advise you to replace the values on the same line to avoid errors on the graph sheet.

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.

If you have troubles with our kit, do not hesitate to contact us by phone or email:

Yannick FOURNIER
Sales engineer
9, avenue de l'Europe - Cap Alpha
34 830 CLAPIERS (FRANCE)
Phone: +33 (0)6 33 64 42 29
Phone: +33 (0)9 67 39 35 20
Email: y.fournier@gl-biocontrol.com

Clément FAYE
Research engineer
9, avenue de l'Europe - Cap Alpha
34 830 CLAPIERS (FRANCE)
Phone: +33 (0)6 72 70 46 98
Phone: +33 (0)9 67 39 35 20
Email: c.faye@gl-biocontrol.com

Controls

Control of the luminometer contamination

a) Test:

- Fix an empty test tube to the tube holder,
- Place it in the luminometer and press the ENTER button,
- The result should be less or equal to 2 RLU.

b) Protocol to be followed in case of contamination:

With a cotton swab, wipe the internal surfaces of the measurement chamber.

Control of the reagent contamination

a) Test:

- In a test tube, put 2 drops of DENDRIDIAG® BF and 4 drops of **EXTRACTANT**,
- Fix the test tube to the tube holder,
- Place it in the luminometer and press the ENTER button,
- The result should be less or equal to 5 RLU.

b) Protocol to be followed in case of contamination:

Discard the contaminated reagents and select a new bottle of DENDRIDIAG® BF and of **EXTRACTANT**.

Control of the reagent efficiency

a) Test:

- 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 the test tube to the tube holder,
- Properly homogenize the tube,
- Place it in the luminometer and press the ENTER button,
- For a good efficacy of the reagents, the result should be higher than 130 RLU.

b) Protocol to be followed in case of degradation:

Discard the reagents and select a new bottle of DENDRIDIAG® BF and of **EXTRACTANT.**

F.A.Q.

GENERAL POINTS ON ATP AND DENDRIDIAG® KITS

What is ATP?

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.

What do I measure with ATP-metry?

In water, quantifying ATP equates to quantifying total microorganisms (or total flora). ATP-metry is a biomolecular technique, based on bioluminescence. The measurement is done using a luminometer.

What do I measure with the DENDRIDIAG® kits?

With DENDRIDIAG® kits and filtration, only intracellular ATP is measured. It corresponds to the ATP found inside the living cells representative of living bacteria.

Extracellular ATP is also found in sample as a free molecule in the sample. It comes from dead or dying microorganisms. Filtration eliminates free ATP. Without filtration, total ATP is measured: intracellular and extracellular ATP.

Which microorganisms are lysed by the DENDRIDIAG® reagent?

DENDRIDIAG® kits preferentially lyses bacteria, cyanobacteria and amoeba. For total lysis of all microorganisms (fungi, yeast, algae…) consult GL BIOCONTROL.

At what temperature should I use the reagents?

To ensure a maximal enzyme efficiency, DENDRIDIAG® reagent and **STANDARD 1000** must be used at room temperature (18°C - 25°C).

How and how long can I store the reagents?

All ATP-metry dropper bottles (DENDRIDIAG® reagents, **STANDARD 1000** and **EXTRACTANT**) must 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 will be preferentially refrozen or, if not, kept refrigerated (between 3 and 8°C) for 8 consecutive weeks.

Stored at room temperature, the reagents are stable less than one week.

How and how long can I keep the plastic consumables?

Plastic consumables must be stored in a dry area at room temperature. Their expiration date is displayed on their individual packaging.

I forgot the DENDRIDIAG® reagent at room temperature. What should I do?

For good stability of the kit, all the reagents must be stored in a freezer (-18°C). If you forgot the reagents at room temperature for a few days, you can perform a control of the reagent efficiency. To do so, refer to the paragraph ***Control of the reagent efficiency*** page 14.

In which areas of application can use the DENDRIDIAG® kits?

Industrial Water (IW): cooling system, process water circuit, water supply system for industrial purposes…

Sanitary Water (SW): drinking water supply system, water network of spa facilities…

Ultra-Pure Water (UPW): water loop system for medical, pharmaceutical or microelectronics use, water networks under microbiological control…

Surface (BF): food processing, cooling tower, water supply system, pools…

Air (AIR): aeraulic network, hospitals, offices, high risks industries like composting, methanation, farming…

OPERATING MODE

What surface dimension should I sample?

Our kit is supplied with a sampling template which defines a surface of 20 cm². With this sampling template, you get a good reproducibility and a significant sampling of the surface studied which leads to reliable results. Nevertheless, if you want to increase the measurement sensitivity, you can perform two sampling of the same surface by moving the sampling template. Always write down the surface sampled.

LUMINOMETER

My tube holder for PD30 luminometer is dirty. How to clean it?

An excess of foam or a bubble in the upper part of the tube can dirty the tube holder. In this case, you can use a paper towel impregnated with alcohol 70% of water to clean the tube holder.

I forgot to write down the R1 and R2 values.

It is possible to retrieve the results in RLU in the luminometer. To do so, turn on the luminometer and after the 10 seconds of calibration, press the up arrow to get the last values obtained.

Luminometer PD30 rings and does not complete calibration.

PD30 calibration is done when the luminometer is empty and the cap correctly closed. If the luminometer rings, make sure the tube holder is out and the cap is well closed.

Luminometer C110 displays « OVERSCALE ».

Luminometer C110 is very sensitive. The message “OVERSCALE” is displayed when the sample is strongly contaminated and the luminometer cannot measure the RLU. If you analyze liquids, restart the measurement by filtrating 1/10th of the volume.

RESULT INTERPRETATION

Which unit should I use to express my results?

Picogram ATP per square centimeter (pgATP/cm²) is the real unit.

For a better understanding of the results, it is possible to use the unit equivalent bacteria per square centimeter (eq.bact./cm²) using the scientific consensus 1 pgATP ≈ 1 000 bacteria. This result is not rigorously true because the ATP concentration varies from microorganism to microorganism and also differs following the metabolic state of the bacteria.

Why express my results in LOG?

Results are often expressed in LOG to simplify the interpretation. Indeed, we consider that two results are significantly different if there is a difference of 1 LOG between the two values.

The following table shows you the conversion of eq.bact./cm² in LOG:

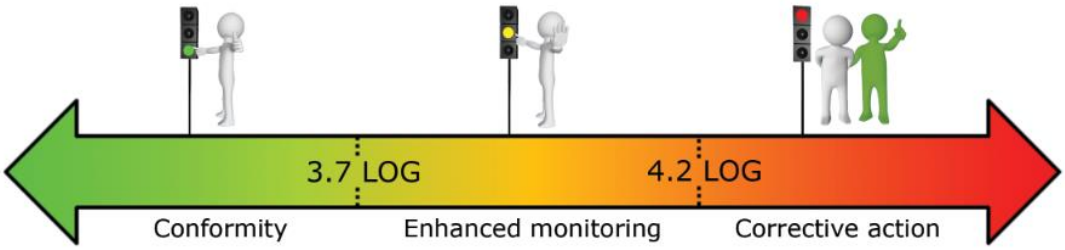
eq.bact./cm²	LOG(eq.bact./cm²)
10	1
100	2
1 000	3
10 000	4
100 000	5

The Excel table automatically gives you this value.

What reference limits should I use for my surface analysis?

Based on our experiment, we established the following warning and alarm thresholds:

Operating swimming pools (in LOG/cm²):



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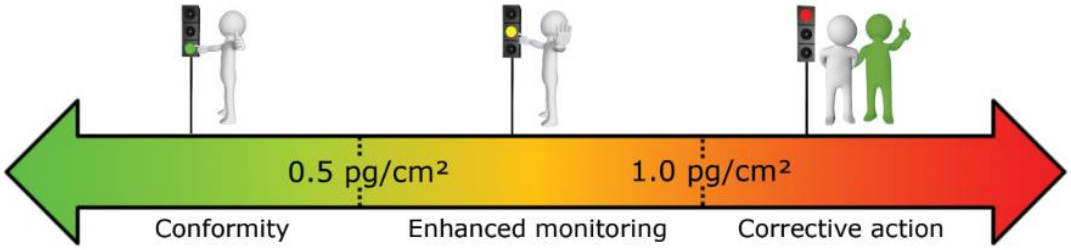

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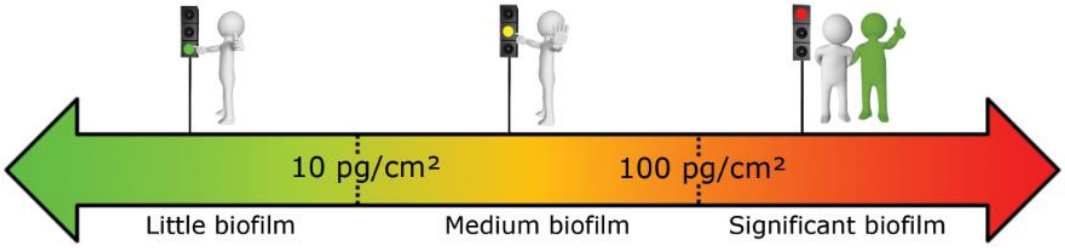
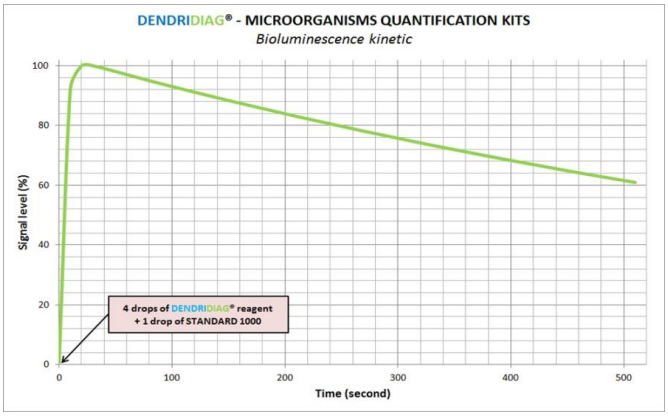


These thresholds should be refined based on the first results obtained on your network.

Why is the R2 value inferior to the R1 value?

Signal loss was estimated to 6% to 8% per minute. If measurement takes a long time and there are several minutes between ATP extraction and reading of R2, standardization will happen during the signal degrowth phase. It is often observed on sample highly contaminated.

We advise you to restart the analysis by sampling a smaller surface.



Why the message "Low sensitivity. If necessary, sample a larger surface." Is 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).

Why the message "Control the mixing of the STANDARD 1000, the temperature and condition of the reagents." Is 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).

Why the message "Sample highly contaminated. If necessary, sample a smaller surface." is displayed in the Excel file.

The ATP concentration of the sample is too high. Restart the analysis by sampling a smaller surface.

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
Phone: +33 (0)9 67 39 35 20
Email: y.fournier@gl-biocontrol.com

Clément FAYE
Research engineer
9, avenue de l'Europe - Cap Alpha
34 830 CLAPIERS (FRANCE)
Phone: +33 (0)6 72 70 46 98
Phone: +33 (0)9 67 39 35 20
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