DENDRIDIAG® UPW

OPERATING MODE FOR QUANTIFICATION OF TOTAL FLORA IN ULTRA-PURE WATER BY ATP-METRY

- LUMINOMETRE KIKKOMAN C110 -

VERSION: V2018-11

GL BIOCONTROL – Company specialized in bioenvironmental risk management of water
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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.

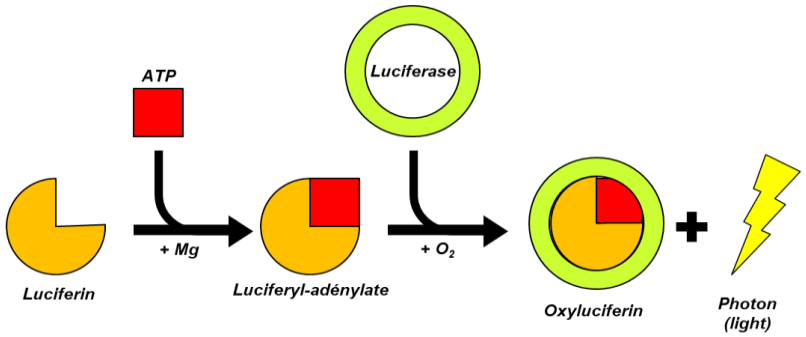
GL BIOCONTROL offers everything you need to quantify total flora in waters, on surfaces and in the air by **ATP-metry**: measurement kits **DENDRIDIAG®**.

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.

In water, 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 liter**. The total flora, expressed in equivalent bacteria per liter, 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 water microbial quality is caused by one or all of the following: untreated makeup water, low quality network materials, dubious operating procedures. There is no definitive solution to eradicate microbiological issues. Only an approach starting with the **observation** of the network 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 in water networks require that the facility manager uses **indicators** to follow and anticipate a microbiological shift in their water networks in order to avoid contaminants such as *Legionella* or *Pseudomonas*.

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:

- **Manage biofouling of your osmosis membrane:** anticipate biofouling, improve health risk management (e.g. for dialysis), avoid production shutdown.
- **Monitor your network:** adapt treatment strategy, reduce production defects and non-quality costs, reduce costs due to production shutdown.
- **Assess operating procedure efficiency:** validate efficiency of cleaning (bio-dispersant), draining or rinsing, disinfection (biocide), avoid downtime and optimize manpower.
- **Identify the critical points of the network:** determine critical points in real time, detect a network component producing biomass, highlight malfunctions.

Protocol for quantification of total flora (sampling from a bottle)

Equipment needed

Kit of reagents for 60 measurements

Product	Quantity	
Dropper bottle DENDRIDIAG® UPW	6	
Dropper bottle **STANDARD 1000**	1	

*Reagents (**DENDRIDIAG® UPW** 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 60 measurements

Product	Quantity	
Single-use filtration syringes of 10ml	60	
Single-use filters 0.45µm pore size	60	
Sterile extension tubes luer-lock	60	
Sterile graduated pipettes 1 ml	60	

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Disposable sterile polypropylene test tubes	60	

Plastic consumables must be stored in a dry location at room temperature. Their use-by-date is displayed on their individual plastic packaging (filter, syringe and extension tube).

Equipment

Product	Quantity	
Luminometer KIKKOMAN C110 or equivalent	1	
Vacuum pump	1	
Laminar flow cabinet	1	
Fridge (about 3 to 8°C) *	1	
Freezer (about -18°C) *	1	

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

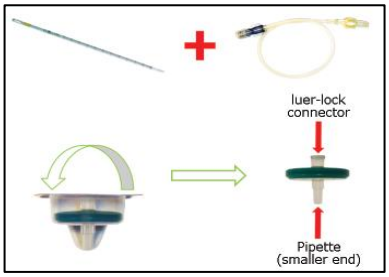
Operating mode

Phase 1: installation

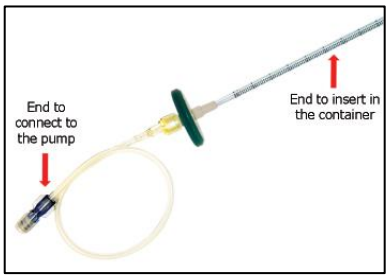
1. Under the laminar flow cabinet, thaw a dropper bottle of each reagent (**DENDRIDIAG® UPW** and **STANDARD 1000**). Bring them to room temperature (above 18°C),
2. Prepare the plastic consumables (syringe, filter, extension tube, pipette and test tube),
3. Turn on the luminometer,
4. In the menu, select "Standard Mode" and make sure the apparatus displays « *Ready* »,
5. Put on disposable gloves,

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

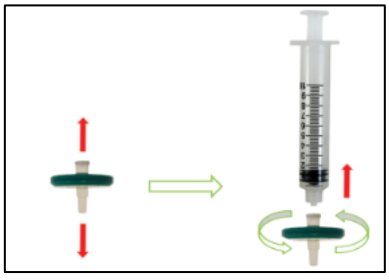
Phase 2: water sampling



1. Open the cap of the filter packaging (do not discard the plastic packaging),
2. Open the pipette on the tip side,
3. Take the extension tube out of its package being careful not to touch the end parts of the component,
4. Connect a pipette on the filter (smaller end),
5. Connect the extension tube on the filter,
6. Connect the extension tube on the vacuum pump,



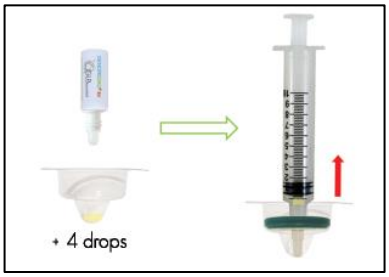
7. Insert the assembly (on the pipette side) in the container and suck up the sample volume desired (around 1000 ml). Do not dry the filter,
8. Write down the volume filtered,
9. Take the syringe out of its packaging being careful not to touch the end part, and suck up 4 ml of air,



10. Disconnect the extension tube and the pipette from the filter without touching the end parts,
11. Screw the syringe on the filter.
12. Slightly push on the piston until the filter grooves are visible to remove the dead volume of water remaining. Stop the pressure to avoid breaking the membrane.

Phase 3: quantification of total flora

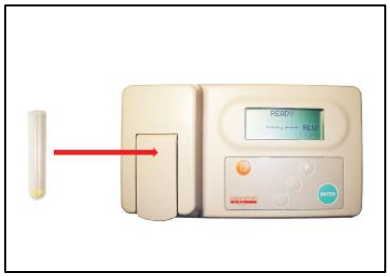
13. Make sure the reagent DENDRIDIAG® UPW is close to room temperature (between 18°C and 25°C) and put 4 drops of DENDRIDIAG® UPW in the bottom of the plastic packaging,
14. Place the filter tip in the bottom of the filter plastic packaging,
15. Suck up all the reagent DENDRIDIAG® UPW through the filter at once. Maintain the depression inside the syringe,



From this step, no break time is allowed.

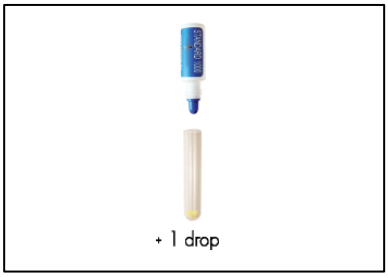
16. By constant pressure on the piston, push the liquid out of the syringe into the test tube until a white foam comes out. The filter tip should touch the internal surface of the test tube so the liquid runs along the wall to the bottom. Stop the pressure as soon as the foam comes out.

17. Place the tube in the luminometer and press the ENTER button to start measurement,
18. After 10 seconds of measurement, write down the R1 result in RLU (Relative Light Unit).

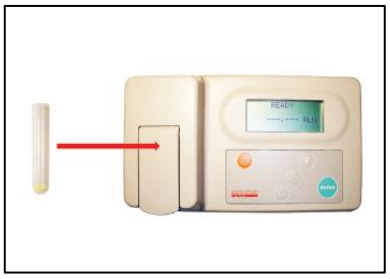


If the luminometer displays "OVERSCALE", the high limit of quantification is exceeded. Restart the protocol with a lower volume of sample (about 1/10th).

19. Immediately, get the test tube out of the luminometer,
20. Add one drop of **STANDARD 1000** in the middle of the test tube. In case the foam forms a barrier in the upper part of the tube, tap the tube on a flat surface to get the foam down, the add the drop of **STANDARD 1000**. When adding the drop, the dropper bottle should not touch the tube.



21. Correctly homogenize the mix by tapping the tube on a flat surface,
22. Place the test tube in the luminometer and press the ENTER button,
23. After 10 seconds of measurement, write down the R2 result in RLU.



If the luminometer displays "OVERSCALE", the high limit of quantification is exceeded. Restart the protocol with a lower volume of sample (about 1/10th).

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Protocol for quantification of total flora (from a sampling valve)

Equipment needed

Kit of reagents for 60 measurements

Product	Quantity	
Dropper bottle DENDRIDIAG® UPW	6	
Dropper bottle **STANDARD 1000**	1	

*Reagents (**DENDRIDIAG® UPW** 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 60 measurements

Product	Quantity	
Single-use filtration syringes of 10 ml	60	
Single-use filters 0.45µm pore size	60	
Sterile luer-lock connectors	60	
Disposable sterile polypropylene test tubes	60	

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Plastic consumables must be stored in a dry location at room temperature. Their use-by-date is displayed on their individual plastic packaging (filter, syringe and extension tube).

Equipment

Product	Quantity	
Luminometer KIKKOMAN C110 or equivalent	1	
Laminar flow cabinet	1	
Fridge (about 3 to 8°C) *	1	
Freezer (about -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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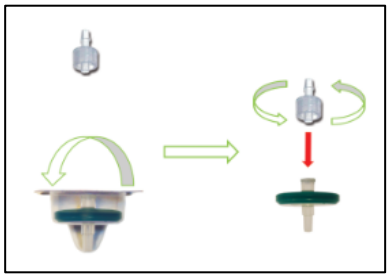
Operating mode

Phase 1: installation

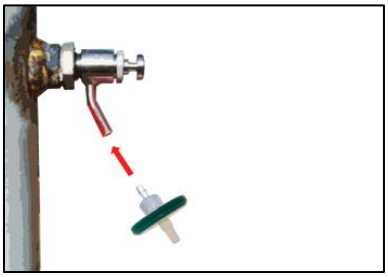
1. Under the laminar flow cabinet, thaw a dropper bottle of each reagent (**DENDRIDIAG® UPW** and **STANDARD 1000**). Bring them to room temperature (above 18°C),
2. Prepare the plastic consumables (syringe, filter, luer-lock connector and test tube),
3. Turn on the luminometer,
4. In the menu, select "Standard Mode" and make sure the apparatus displays « *Ready* »,
5. Put on disposable gloves,

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

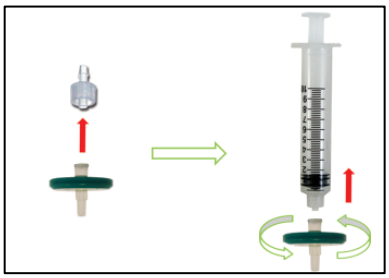
Phase 2: water sampling



1. Open the cap of the filter packaging (do not discard the plastic packaging),
2. Connect the luer-lock connector on the filter being careful not to touch the end parts of the component,
3. Flush the sampling point for 20 seconds, then stop the flow,
4. Connect the filter-connector to the PEMS II valve,

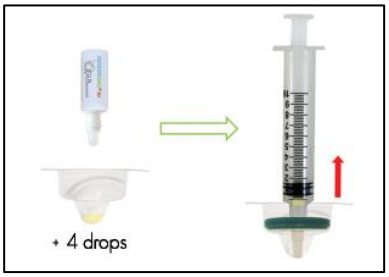


5. Open the valve and filter one liter of water. Measure the volume filtered by filling a waste container,
6. Write down the volume filtered,
7. Take the syringe out of its packaging being careful not to touch the end part, and suck up 4 ml of air,
8. Disconnect the luer-lock connector without touching the end parts of the filter,



9. Screw the syringe on the filter.
10. Slightly push on the piston until the filter grooves are visible to remove the dead volume of water remaining. Stop the pressure to avoid breaking the membrane.

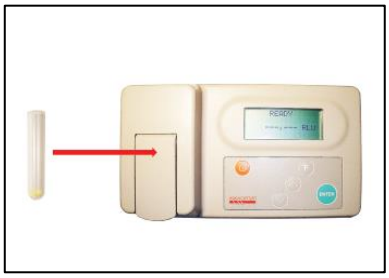
Phase 3: quantification of total flora



11. Make sure the reagent DENDRIDIAG® UPW is close to room temperature (between 18°C and 25°C) and put 4 drops of DENDRIDIAG® UPW in the bottom of the plastic packaging,
12. Place the filter tip in the bottom of the filter plastic packaging,
13. Suck up all the reagent DENDRIDIAG® UPW through the filter at once. Maintain the depression inside the syringe,

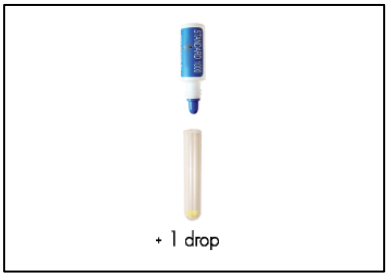
From this step, no break time is allowed.

14. By constant pressure on the piston, push the liquid out of the syringe into the test tube until a white foam comes out. The filter tip should touch the internal surface of the test tube so the liquid runs along the wall to the bottom. Stop the pressure as soon as the foam comes out.
15. Place the tube in the luminometer and press the ENTER button to start measurement,
16. After 10 seconds of measurement, write down the R1 result in RLU (Relative Light Unit).

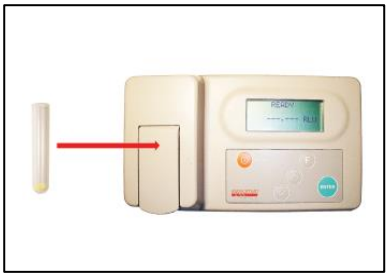


If the luminometer displays "OVERSCALE", the high limit of quantification is exceeded. Restart the protocol with a lower volume of sample (about 1/10th).

17. Immediately, get the test tube out of the luminometer,
18. Add one drop of **STANDARD 1000** in the middle of the test tube. In case the foam forms a barrier in the upper part of the tube, tap the tube on a flat surface to get the foam down, the add the drop of **STANDARD 1000**. When adding the drop, the dropper bottle should not touch the tube.



19. Correctly homogenize the mix by tapping the tube on a flat surface,
20. Place the test tube in the luminometer and press the ENTER button,
21. After 10 seconds of measurement, write down the R2 result in RLU.



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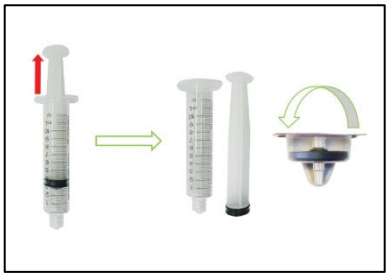
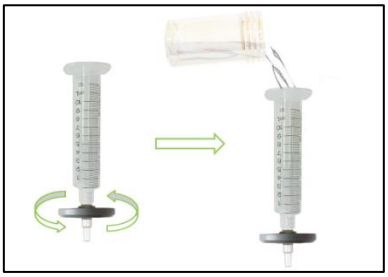
Interpretation of results

Measurement of the negative control (Rbm)

In order to get the most sensitive measurement possible, it is required to subtract the background noise of the protocol. To do so, perform an ATP measurement on sterile water:

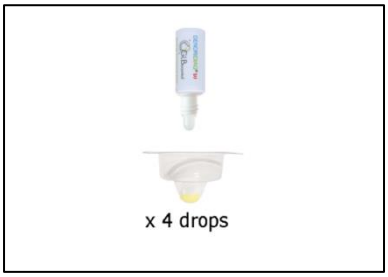
1. Take the syringe out of its package,
2. Remove the syringe piston and put it down, being careful not to touch the lab bench with the black part,
3. Open the cap of the filter packaging (do not discard the plastic packaging),
4. Firmly screw the syringe on the filter to ensure it is watertight,
5. Pour the sample vial content into the syringe,
6. Insert the piston inside the syringe. Filter all the sample until the filter grooves are visible once again. Do not push the piston at the bottom of the syringe to avoid drying the membrane.

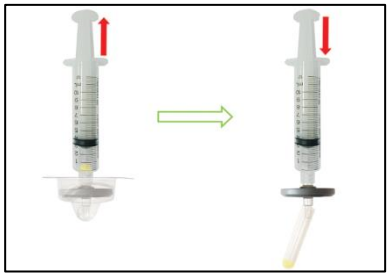




Make sure the reagent is close to room temperature (>18°C). Warm up the reagent in your hand if necessary.

7. Put 4 drops of DENDRIDIAG® UPW in the bottom of the plastic packaging,
8. Place the filter tip in the bottom of the filter plastic packaging,
9. Suck up all the reagent DENDRIDIAG® UPW through the filter. Maintain the depression inside the syringe,
10. By constant pressure on the syringe piston, push the liquid out of the syringe into test tube until a white foam comes out,
11. Place the tube in the luminometer and press the ENTER button to start measurement,
12. After 10 seconds of measurement, write down the Rbm result (in Relative Light Unit).





Calculation of total biomass quantity

The intracellular ATP concentration is expressed in picogram ATP per liter. 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/l):

$$[\text{ATP}] = \frac{\text{R1 - Rbm}}{\text{STANDARD x V}}$$

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,

Rbm = result of the « Blank » in RLU,

V = volume filtered in liter.

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

If the result obtained is lower or equal to 0, the signal is in the background noise. The result of the analysis is: < 100 bacteria/l, below the limit of quantification of the method.

Data managing with Excel

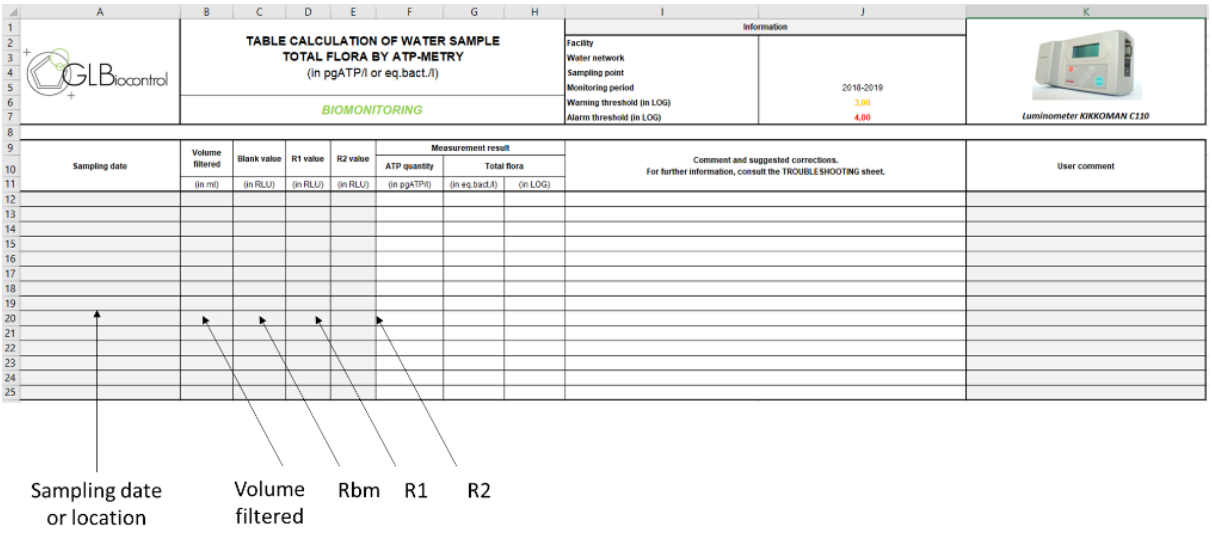
An Excel file is supplied by GL BIOCONTROL to automatically perform the calculations presented above. The file must be used to monitor your facility and interpret the values obtained.

After each analysis, sampling date or location, volume filtered, Rbm, 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 liter (pg ATP/l),
- total flora in **equivalent bacteria** per liter (eq.bact./l),
- total flora in **logarithm** per liter (LOG eq.bact./l).

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



In case you **monitor your water network 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 water quality.*

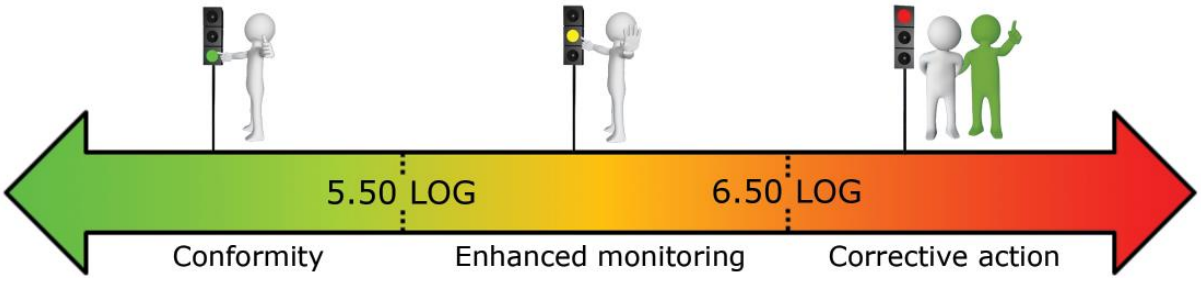
In case you **do a cartography of your water network**, fill in the « CARTOGRAPHY » sheet. Each line corresponds to a point of the network to control. A graph is automatically drawn in the « GRAPH CARTOGRAPHY » sheet. If you want to perform several cartographies, you have to duplicate the sheet or replace the previous values.

⇨ *It is advisable to perform a measurement upstream and downstream of each key component of the water network.*

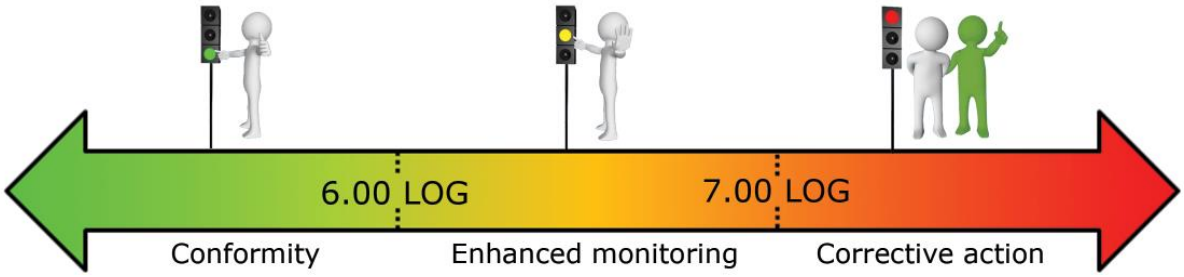
Result interpretation

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

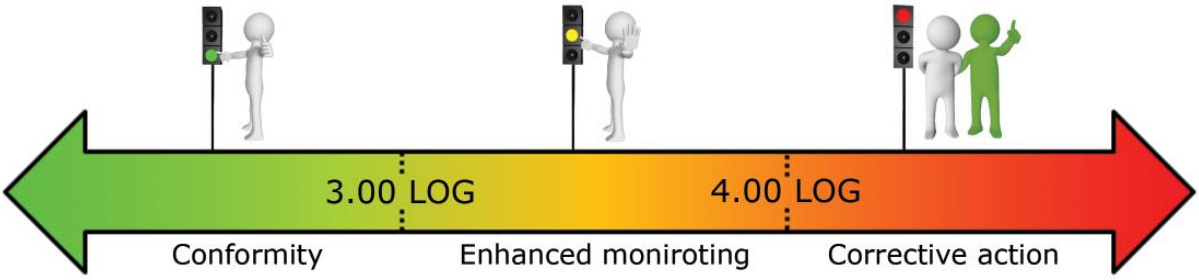
Tap water (in LOG eq.bact./l):



Soft water (in LOG eq.bact./l):



Ultra-pure water (in LOG eq.bact./l):



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. 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 2 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 of the reagents. Control the mixing of the STANDARD 1000, the temperature and condition of the reagents." displayed in the Excel file.	The reagent DENDRIDIAG® UPW is not sufficiently active (out-of-date, degraded or too cold) to obtain high sensitivity. 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 22).
"Blank value too high. Verify the microbial quality of the sterile water and consumables." displayed in the Excel file.	The sterile water, the consumables or the DENDRIDIAG® UPW reagent are contaminated. Perform a ***Control of the luminometer contamination*** and/or a ***Control of the reagent contamination*** (cf. page 22). If the problem remains, use a new sterile water bottle.
Too much foam in the test tube.	During manipulation, lean the test tube so the reagent runs along the tube wall. Stop the pressure as soon as the foam comes out of the syringe. Properly homogenize the test tube after standard addition by tapping the bottom of the tube 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. To do so, stop the vacuum pump as soon as the container is empty.

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I cannot filter all my sample.

When sampling, the pipette probably went out of the water sample and air was sucked up. In this case, the filter is no longer permeable to water.

If you have filtered enough water, write down the volume sampled and continue the protocol.

If you have not filtered enough water, replace the sampling assembly (filter, pipette and extension tube) and restart the protocol.

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

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Controls

Control of the luminometer contamination

a) Test:

- Insert an empty test tube in the luminometer,
- Close the cap and press the ENTER button,
- The result should be less or equal to 5 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® UPW**,
- Insert the test tube in the luminometer,
- Close the cap and press the ENTER button,
- The result should be less or equal to 50 RLU.

b) Protocol to be followed in case of contamination:

Discard the contaminated reagent and select a new bottle of **DENDRIDIAG® UPW.**

Control of the reagent efficiency

a) Test:

- In a test tube, put 2 drops of **DENDRIDIAG® UPW** and 1 drop of **STANDARD 1000** (reagent temperature must be above 18°C),
- Properly homogenize the tube
- Insert the test tube in the luminometer,
- Close the cap and press the ENTER button,
- For a good efficacy of the reagents, the result should be higher than 50 000 RLU.

c) Protocol to be followed in case of degradation:

Discard the reagent and select a new bottle of **DENDRIDIAG® UPW**.

Control of the battery

When starting the luminometer, the level of the battery is displayed. Battery status is defined from 1 to 5. If the luminometer displays « BATTERY 1 », plug the device before continuing the measurements.

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 19.

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 volume of sample should I filtrate?

By default, we advise you to filtrate:

- **10 ml** for the IW kit
- **50 ml** for the SW kit
- **100 ml** for the UPW kit

It is necessary to filtrate a representative volume of sample in order to get a better reliability of the results. Anyway, always write down the volume filtrated for each sample.

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. To do so, stop the vacuum pump as soon as the container is empty.

I cannot filter all my sample.

When sampling, the pipette probably went out of the water sample and air was sucked up. In this case, the filter is no longer permeable to water.

If you have filtered enough water, write down the volume sampled and continue the protocol.

If you have not filtered enough water, replace the sampling assembly (filter, pipette and extension tube) and restart the protocol.

LUMINOMETER

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 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.

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RESULT INTERPRETATION

Which unit should I use to express my results?

Picogram ATP per liter (pgATP/l) is the real unit.

For a better understanding of the results, it is possible to use the unit equivalent bacteria per liter (eq.bact./l) 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./l in LOG:

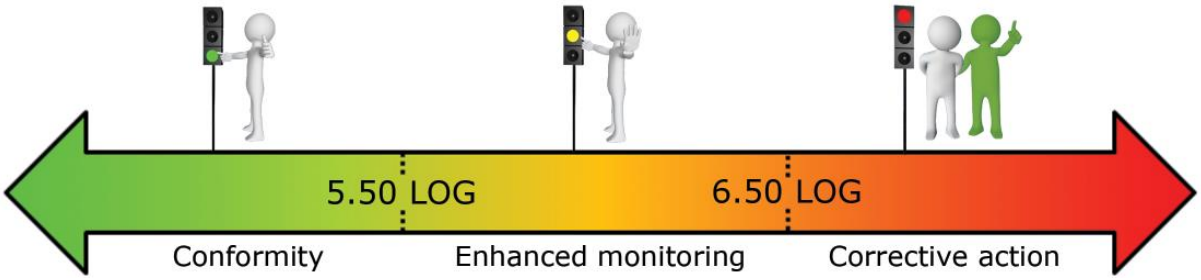
eq.bact./l	LOG(eq.bact./l)
10	1
100	2
1 000	3
10 000	4
100 000	5

The Excel table automatically gives you this value.

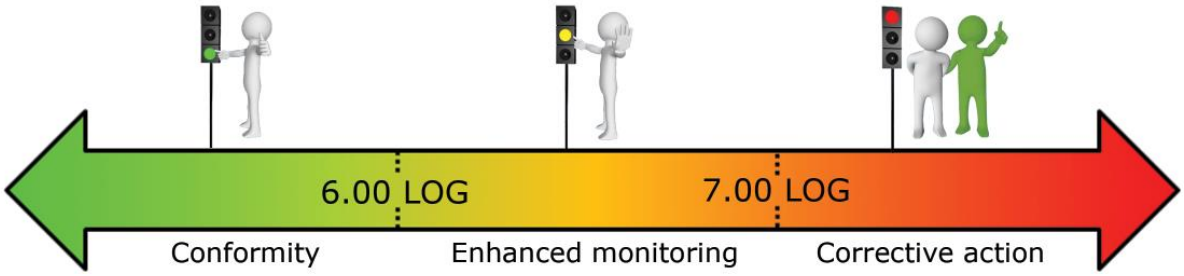
What reference limits should I use for my water analysis?

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

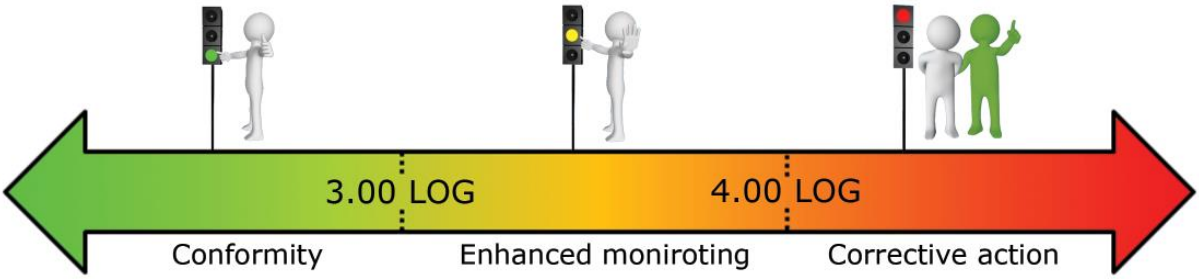
Tap water (in LOG eq.bact./l):



Soft water (in LOG eq.bact./l):



Ultra-pure water (in LOG eq.bact./l):

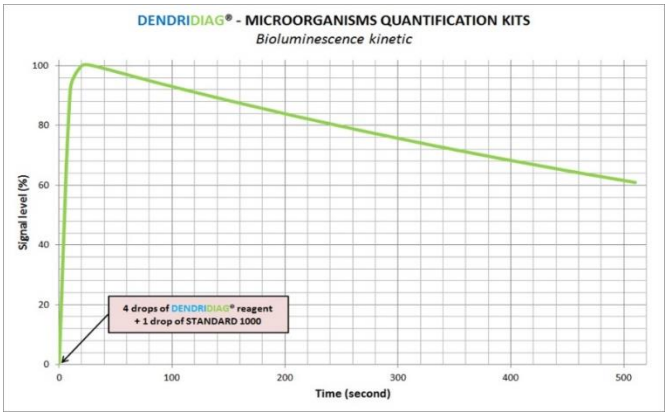


These thresholds should be refined based on the first results obtained on your network.

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 filtrating a smaller volume of water.



Why the message "Low sensitivity of the reagents. Control the mixing of the STANDARD 1000, the temperature and condition of the reagents." displayed in the Excel file?

The reagent **DENDRIDIAG® UPW** is not sufficiently active (out-of-date, degraded or too cold) to obtain high sensitivity.

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 22).

Why the message "Blank value too high. Verify the microbial quality of the sterile water and consumables." displayed in the Excel file?

The sterile water, the consumables or the **DENDRIDIAG® UPW** reagent are contaminated.

Perform a ***Control of the luminometer contamination*** and/or a ***Control of the reagent contamination*** (cf. page 22).

If the problem remains, use a new sterile water bottle.

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 – ATP-metry kit for ultra-pure water:

www.gl-biocontrol.com