OPERATING MODE DENDRIDIAG® UPW

Quantification of total flora in ultra-pure water by ATP-metry

- LUMINOMETER KIKKOMAN C110 -

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:

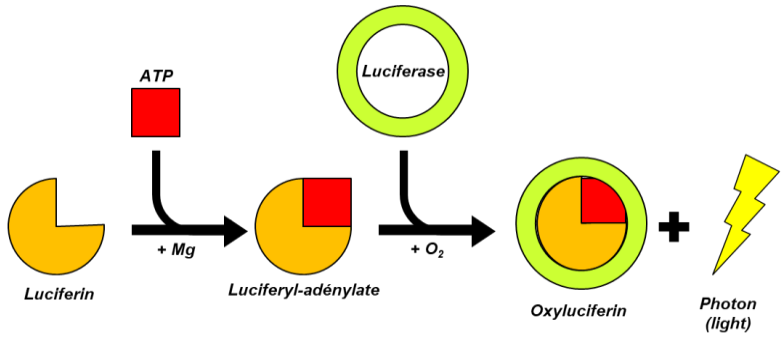
- **Develops** risk management tools (ATP-metry kits for total flora quantification of water or surfaces, DNA extraction purification kits, real time PCR amplification kits…).
- **Uses** methodologies and innovative tools to study the microbial world (qPCR, NGS, ATP-metry…).
- **Studies** ecosystems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

In water, 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).



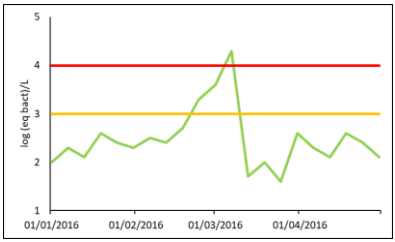
ATP, in the presence of a luciferin/luciferase complex with a catalyst, releases energy in the form of light. By measuring the amount of light emitted using a **luminometer**, we deduce the **quantity of ATP in picogram per liter**. The total flora, expressed in equivalent bacteria per liter, is calculated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

1 picogram ≈ 1 000 bacteria.

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

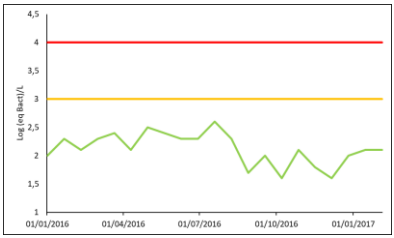
GL BIOCONTROL's ATP-metry

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



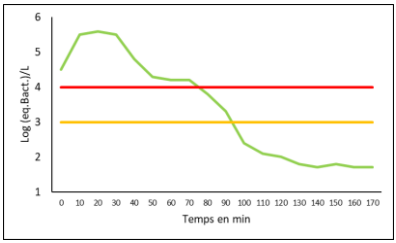
Manage biofouling of your osmosis membrane:

- Anticipate biofouling.
- Improve health risk management (e.g. for dialysis).
- Avoid production shutdown.



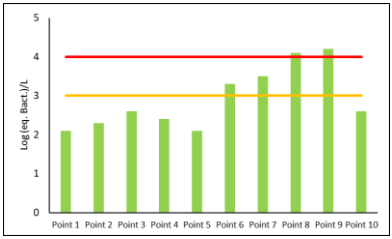
Monitor your network:

- 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 point of the network:

- Determine critical points in real time.
- Detect a network component producing biomass.
- Highlight malfunctions.

Equipment needed

Kit of reagents for 60 measurements

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

Kit of consumables for 60 measurements (sampling from a bottle)

Product	Quantity
Single-use filtration syringes (10ml)	60
Single-use filters 0.45µm pore size	60
Sterile extension tubes luer-lock	60
Sterile graduated pipettes	60
Disposable sterile polypropylene test tubes	60

Kit of consumables for 60 measurements (from a sampling valve)

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

Equipment

Product	Quantity
Luminometer KIKKOMAN C110 or equivalent	1
Electronic vacuum pump (only for sampling from a bottle)	1
Laminar flow cabinet	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 should preferentially be refrozen. Or else, they can be kept refrigerated (between 3 and 8°C) for a maximum of 8 consecutive weeks.*

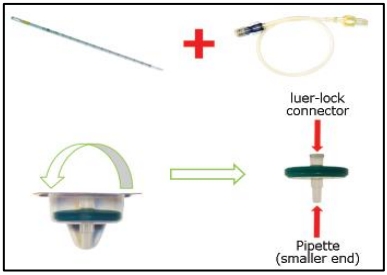
Protocol: sampling from a bottle

Installation

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).

Prepare the plastic consumables (syringe, filter, extension tube luer-lock, graduated pipette and test tube). Turn on the luminometer and select "Standard Mode".

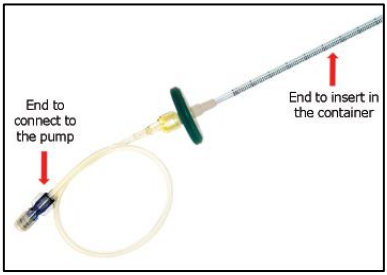
Sampling



Open the cap of the filter packaging (do not discard the plastic packaging). Open the pipette on the tip side.

Take the extension tube out of its package being careful not to touch the end parts of the component.

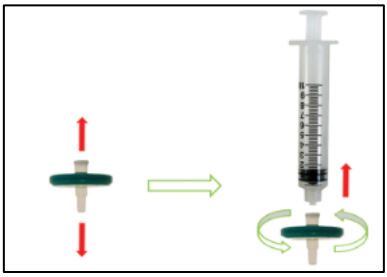
Connect a pipette on the filter (smaller end). Connect the extension tube on the filter.



Connect the extension tube on the vacuum pump.

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.

Write down the volume filtered.



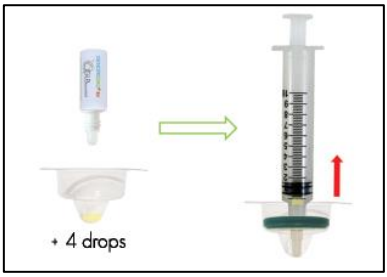
Take the syringe out of its packaging and suck up 4 ml of air.

Disconnect the extension tube and the pipette from the filter without touching the end parts.

Screw the syringe on the filter. Slightly push on the piston until the filter grooves are visible to remove the dead volume of water remaining.

Quantification of total flora

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



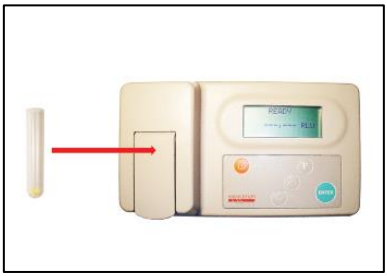
Put 4 drops of DENDRIDIAG® UPW in the bottom of the plastic packaging.

Place the filter tip in the bottom of the plastic packaging. Suck up all the reagent through the filter. Maintain the depression inside the syringe.

From this step, no break time is allowed.

By constant pressure on the syringe piston, push the liquid out of the syringe into test tube until a white foam comes out.

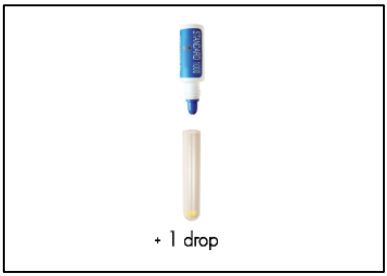
Stop the pressure as soon as the foam comes out. A too strong or long pressure on the piston will create a foam between the reagent and the top of the test tube. This should be avoided to have a correct mixing of the **STANDARD 1000** with the reagent.



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

Write down the R1 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).



Immediately, get the test tube out of the luminometer.

Add one drop of **STANDARD 1000** in the middle of the test tube.

Correctly homogenize the mix.

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.

Place the test tube in the luminometer and press the ENTER button.

Write down the R2 result (in RLU).

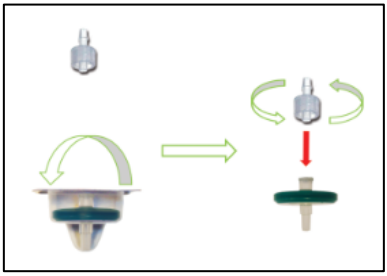
Protocol: from a sampling valve

Installation

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).

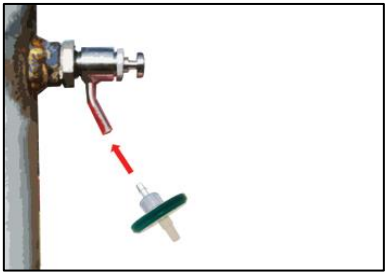
Prepare the plastic consumables (syringe, filter, luer-lock connector and test tube). Turn on the luminometer and select “Standard Mode”.

Sampling



Open the cap of the filter packaging (do not discard the plastic packaging).

Connect the luer-lock connector on the filter being careful not to touch the end parts of the component.

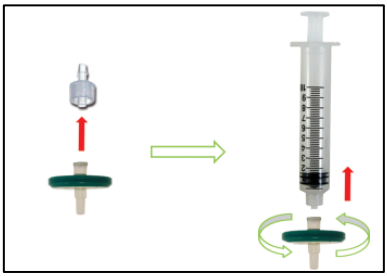


Flush the sampling point for 20 seconds, then stop the flow.

Connect the filter-connector to the PEMS II valve.

Open the valve and filter one liter of water. Measure the volume filtered by filling a waste container.

Write down the volume filtered.



Take the syringe out of its packaging and suck up 4 ml of air.

Disconnect the luer-lock connector without touching the end parts of the filter.

Screw the syringe on the filter. Slightly push on the piston until the filter grooves are visible to remove the dead volume of water remaining.

Quantification of total flora

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

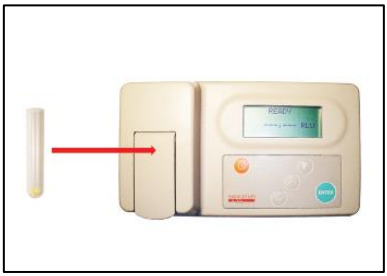
Put 4 drops of DENDRIDIAG® UPW in the bottom of the plastic packaging.

Place the filter tip in the bottom of the plastic packaging. Suck up all the reagent through the filter. Maintain the depression inside the syringe.

From this step, no break time is allowed.

By constant pressure on the syringe piston, push the liquid out of the syringe into test tube until a white foam comes out.

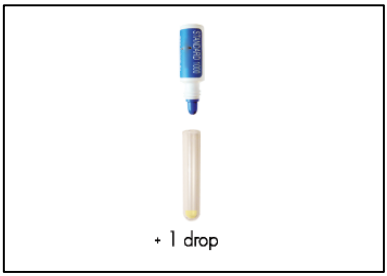
Stop the pressure as soon as the foam comes out. A too strong or long pressure on the piston will create a foam between the reagent and the top of the test tube. This should be avoided to have a correct mixing of the **STANDARD 1000** with the reagent.



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

Write down the R1 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).



Immediately, get the test tube out of the luminometer.

Add one drop of **STANDARD 1000** in the middle of the test tube.

Correctly homogenize the mix.

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.

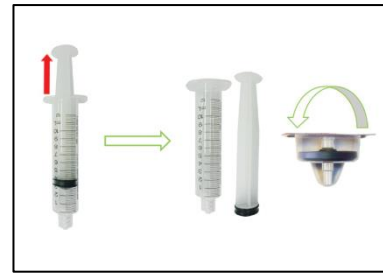
Place the test tube in the luminometer and press the ENTER button.

Write down the R2 result (in RLU).

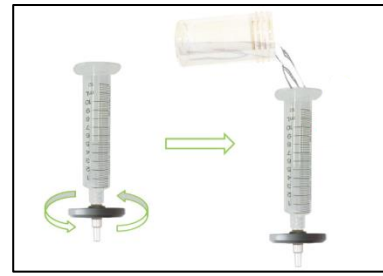
Interpretation of results

Measurement of the negative control (Rbm)

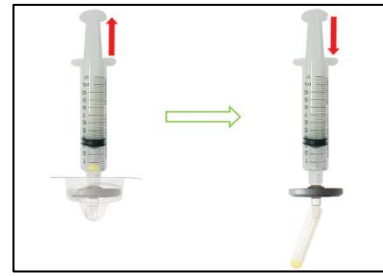
In order to get the most sensitive measurement possible, it is required to subtract the background noise of the protocol. To do so, perform an ATP measurement on sterile water:



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



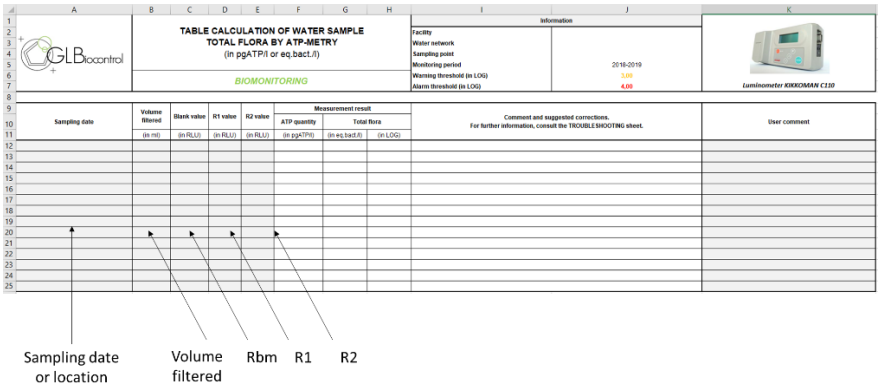
Make sure the reagent is close to room temperature (>18°C). Warm up the reagent in your hand if necessary.



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

Data management with Excel

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

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

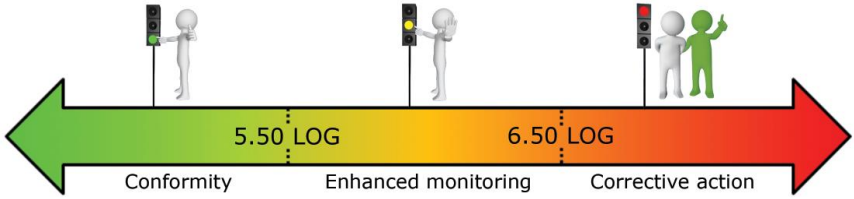
In case you **do a cartography of your water network**, fill in the « CARTOGRAPHY » sheet. A graph is automatically drawn in the « GRAPH CARTOGRAPHY » sheet.

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

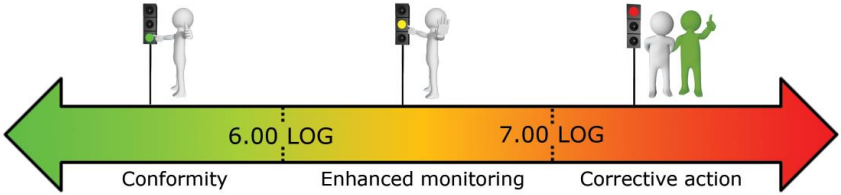
If the Rbm value is higher than R1, the signal is in the background noise. The result of the analysis is: < 100 bacteria/l, below the limit of quantification of the method.

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

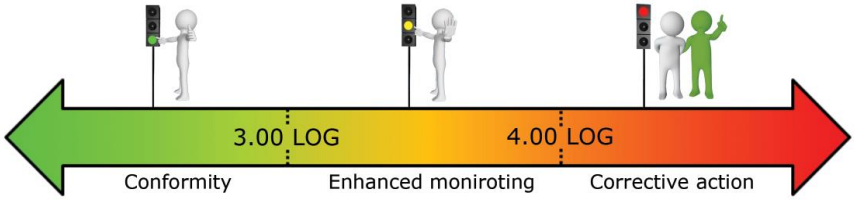
Tap water (in LOG eq.bact./l):



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.

Troubleshooting

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 18).
"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 18). 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.
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.

Controls

Control of the luminometer contamination

- 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. 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® 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. If not, discard the contaminated reagent and select a new bottle of **DENDRIDIAG® UPW.**

Control of the reagent efficiency

- 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. If not, 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.

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

Nicolas FABRE
Technical manager
9, avenue de l'Europe - Cap Alpha
34 830 CLAPIERS (FRANCE)
Phone: +33 (0)6 81 71 31 83
Email: n.fabre@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 – ATPmetry kit for ultra-pure water:

www.gl-biocontrol.com

In addition of the Excel file, the web application **DENDRIDIAG® SOFTWARE** is available on smartphone and tablet at:

https://dendridiag.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