PROCEDURE FOR THE USE AND QUALIFICATION OF THE TOTAL FLORA MEASUREMENT KIT

DENDRIDIAG® UPW

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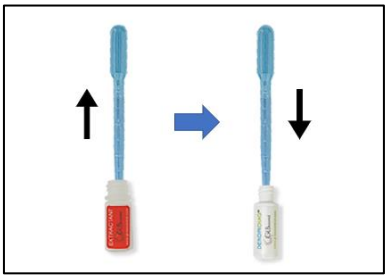
REHYDRATION OF THE DENDRIDIAG® REAGENT



1. Thaw a bottle of **EXTRACTANT** and a dropper-bottle of **DENDRIDIAG® UPW**.
2. Remove the cap of the **DENDRIDIAG® UPW** dropper-bottle.
3. With the sterile transfer pipet supplied, carefully suck up the whole **EXTRACTANT** solution in one time. Avoid the formation of foam.
4. Carefully transfer the solution in the **DENDRIDIAG® UPW** dropper-bottle.
5. Remove the aluminium seal from the white cap. Maintain the opening up.
6. Screw the white cap on the **DENDRIDIAG® UPW** dropper-bottle until you hear “clic”.
7. Leave the reagent at room temperature 10 min before use. To reduce the noise signal to a minimum, leave it to stand 30 min.

After rehydration, the reagents must be kept in the freezer (< -20°C). They can undergo up to 5 freeze-thaw cycles.

SAMPLE MEASUREMENT

Phase 1: installation

1. Under the laminar flow cabinet, thaw a dropper bottle of each reagent (**DENDRIDIAG® UPW** and **STANDARD**). 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.*

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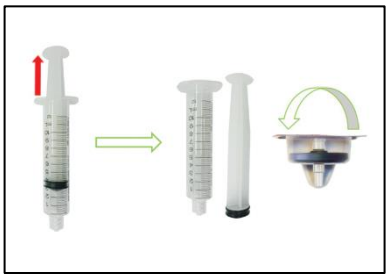
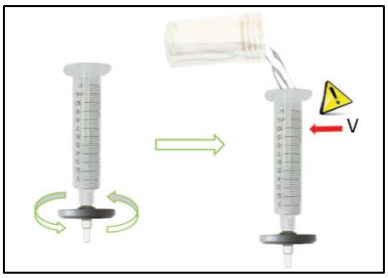
Phase 2: measurement of the negative control (Rbm)

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:

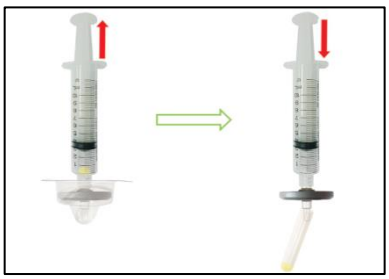


1. Take the syringe out of its package. Remove the syringe piston and put it down, being careful not to touch the lab bench with the black part.
2. Open the cap of the filter packaging (do not discard the plastic packaging).
3. Firmly screw the syringe on the filter to ensure it is watertight.



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).

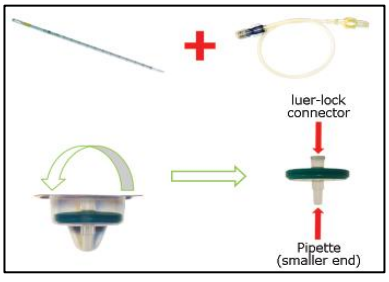


6. Add 4 drops of DENDRIDIAG® UPW in the bottom of the plastic packaging.
7. Place the filter tip in the bottom of the filter plastic packaging. Suck up all the reagent DENDRIDIAG® UPW through the filter. Maintain the depression inside the syringe.
8. By constant pressure on the syringe piston, push the liquid out of the syringe into the test tube until a white foam comes out. Stop the pressure as soon as the 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).

Phase 3: water sampling

To facilitate the sampling, place a 2 or 3-way luer-lock valve at the output of the pump.

1. Open the cap of the filter packaging (do not discard the plastic packaging),
2. Take the extension tube out of its package being careful not to touch the end parts of the component,



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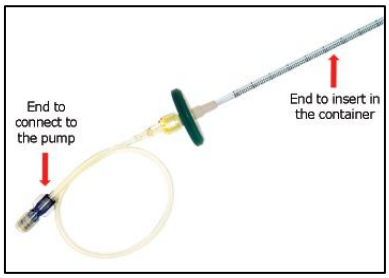
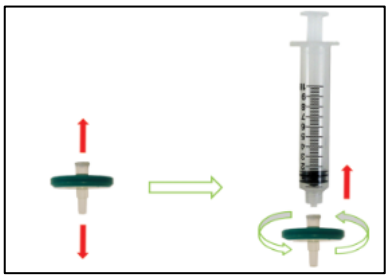
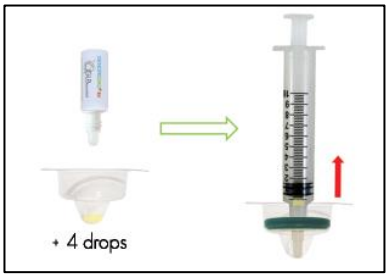
3. Connect the luer-lock female of the extension tube on the vacuum pump (on the 2-way valve),
4. Connect the luer-lock male of the extension tube on the filter,
5. Open the pipette on the tip side,
6. Connect the pipette on the filter (smaller end),
7. Insert the assembly (on the pipette side) in the container,
8. Turn on the vaccum pump and open the 2-way valve,
9. Suck up the sample volume desired (around 1000 ml). Do not dry the filter,
10. Stop the filtration by closing the 2-way valve,
11. Write down the volume filtered,
12. Take the syringe out of its packaging being careful not to touch the end part, and suck up 4 ml of air,
13. Disconnect the pipette and recover the filter without touching the end parts,
14. Screw the syringe on the filter. Do not flush the water remaining on the filter.





Phase 4: quantification of total flora

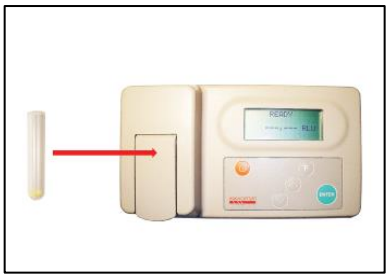
1. Make sure the reagent **DENDRIDIAG® UPW** is close to room temperature (between 18°C and 25°C) and put 4 drops of **DENDRIDIAG® UPW** in the bottom of the plastic packaging,
2. Place the filter tip in the bottom of the filter plastic packaging,
3. Suck up all the reagent **DENDRIDIAG® UPW** through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



From this step, no break time is allowed.

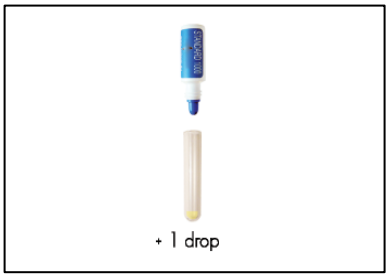
4. By constant pressure on the piston, push the liquid out of the syringe into the test tube until a white foam comes out. The filter tip should touch the internal surface of the test tube so the liquid runs along the wall to the bottom. Stop the pressure as soon as the foam comes out.

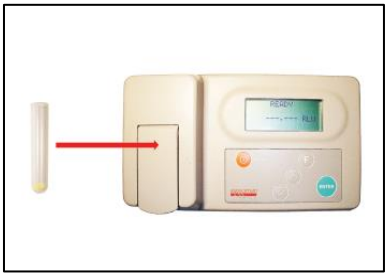
5. Place the tube in the luminometer and press ENTER to start measurement,
6. After 10 seconds of measurement, write down the R1 result in RLU (Relative Light Unit).



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

7. Immediately, get the test tube out of the luminometer,
8. Add one drop of **STANDARD** 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**. When adding the drop, the dropper bottle should not touch the tube.
9. Correctly homogenize the mix by tapping the tube on a flat surface,
10. Place the test tube in the luminometer and press ENTER,
11. 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).

CALCULATION OF TOTAL BIOMASS QUANTITY

Manual calculation

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** in RLU,

STANDARD = value of the standard 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 equivalent bacteria.
For example, 5 pg/l ≈ 5000 eq.bact./l.

If the result obtained (R1 – Rbm) is lower or equal to 0, the signal is in the background noise. The result of the analysis is below the limit of detection of the method.

Data managing with Excel

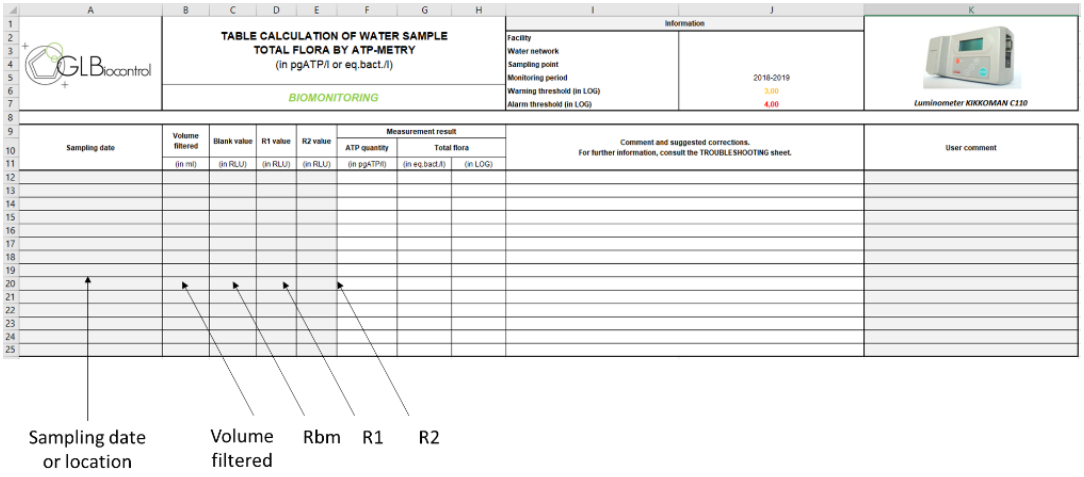
An Excel file is supplied to automatically perform the calculations presented above. The file must be used to monitor your facility and interpret the values obtained.

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** of ATP 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.



EVALUATION OF THE LINEARITY WITH ATP SOLUTIONS

To assess the linearity of DENDRIDIAG kit, the temperature of the ATP solutions and of the reagents must be held constant throughout the tests (around 20°C).

The **STANDARD** reagent contains 1000 pg of ATP in 40 µl.

The **STANDARD** reagent is diluted in a 10-fold serial dilution in sterile water to get 4 concentrations: 1000 pg/40µl – 100 pg/40µl – 10 pg/40µl – 1 pg/40µl.

Preparation of the ATP scale

- Drop 10 drops of **STANDARD** in a sterile tube (labelled QS4).
- Transfer 100 µl of **QS4** into a tube containing 900µl of sterile water (labelled QS3).
- Transfer 100 µl of **QS3** into a tube containing 900µl of sterile water (labelled QS2).
- Transfer 100 µl of **QS2** into a tube containing 900µl of sterile water (labelled QS1).

Measurement

- Transfer 40 µl ATP solution (QS4) into a measurement tube.
- Add 2 drops of **DENDRIDIAG**®.
- Gently mix by tapping the tube on a flat surface.
- Insert the tube in the luminometer.
- Close the lid and press « Enter » to start the measurement.
- Perform the measurement 3 times for each dilution.

Calibration line

Get the calibration line by plotting the quantity of RLU obtained (vertical axis) against the theoretical quantity of ATP (horizontal axis):

$$y = ax + b$$

With y = RLU
x = theoretical pgATP/40µl
a = slope
b = intercept

The correlation coefficient R^2 of the calibration line must be higher than 0.98.

CONTROLS

Control of the luminometer contamination

a) Test:

- Insert an empty test tube in the luminometer,
- Close the cap and press ENTER,
- 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 ENTER,
- 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** (reagent temperature must be above 18°C),
- Mix the tube by tapping on a flat surface,
- Insert the test tube in the luminometer,
- Close the cap and press ENTER,
- 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.