2 MIN UNIT-ORAL®
OPERATING MODE FOR QUANTIFICATION OF TOTAL FLORA IN DENTAL UNIT WATER BY ATP-METRY

- LUMINOMETER KIKKOMAN PD30 -
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1. **INSTITUT CLINIDENT overview**

Founded in July 2003, INSTITUT CLINIDENT is a company that specialized in oral care biology and dental clinic risk management, with an expertise in molecular biology, mass spectrometry and ATP-metry monitoring. Through these areas of expertise, Institut clinident team:

- **Develops & Manufacture** specific kits (ATP-metry kits for total flora quantification, DNA/RNA extraction purification kits, real time PCR amplification kits for Periodontal disease, caries...).
- **Uses** methodologies and innovative tools to study the oral microbial world (qPCR, VOC analysis, ATP-metry...).
- **Studies** the oral and saliva ecosystems.
- **Advises** the actors of the oral care sector on how to use biology in their clinic facilities in order to improve oral treatment, motivate patient and reduce dental clinic environmental risks.
- **Trains** dental professionals on oral biology and microbiological risk management in the clinic.

Although the head office is located in the South of France, our company sells its products and services all over the world. Our clients our divided into various sectors such as private clinics, dental school and university, oral care industry, research institutes.
2. What is ATP-metry?

“ATP-metry is a molecular biological technique, based on bioluminescence phenomena, which measures a quantity of ATP in a sample”

Adenosine triphosphate (ATP) is the major intermediary energy required in most cellular metabolism reactions. It is a cellular metabolism product synthesized in specific organelles called mitochondria which are found in eukaryotes and prokaryotes.

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

3. Why use ATP-metry for microbiological monitoring?

Contamination or degradation of water’s microbiological quality is caused by one or all of the following: untreated water supplies, 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**.

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:

- **Anticipate shifts in your network**: improve health risk management and avoid non-compliance with the regulatory requirement (*Legionella, Pseudomonas...*),
- **Assess operating procedure efficiency**: validation of the efficiency (cleaning, disinfection and water quality),
- **Identify the critical point of the network**: determining critical points and highlighting malfunctions of dental chair unit, measuring microbiological of dental clinic surface and instrumentation, control of sterilization process and microbiological quality of dental equipments.

4. **Protocol for quantification of total flora in sanitary water**

   a. **Reagents**

   - Dropper bottles **2 MIN UNIT-ORAL**®,
   - Dropper bottle **STANDARD 1000**.

   *Reagents (2 MIN UNIT-ORAL® 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 can be kept refrigerated (between 3 and 8°C) for 8 consecutive weeks.*
b. Consumables
- Single-use sterile 60ml sample vials,
- Single-use filtration syringes of 50ml,
- Single-use filters 0.45µm pore size
- Disposable sterile polypropylene test tubes

Plastic consumables should be stored in a dry place at room temperature. Their expiry date is written on their unit package (sample vials, filters and syringes).

c. Equipment needed
- Luminometer KIKKOMAN PD30,
- Tube holder for PD30,
- Freezer (-18°C),
- Fridge (3 to 5°C).
d. Procedure

Phase 1: installation

- On a flat and clean surface, prepare the luminometer, a dropper bottle of 2 MIN UNIT-ORAL® and STANDARD 1000 with the consumables necessary,
- Turn on the luminometer (measurement chamber closed) and wait 10 seconds for the device calibration.

At this moment, the reagents 2 MIN UNIT-ORAL® and STANDARD 1000 must be at room temperature (between 15°C and 25°C) to ensure maximal efficiency of the enzyme.

Phase 2: quantification of the total flora in a water sample

The first step aims to filtrate the water sample in order to concentrate the microorganisms on the filter (0.45µm pore size).

- Take the syringe out of its package being careful not to touch the bottom part,
- Remove the cap of the filter packaging (do not discard the plastic packaging),
- Remove the syringe piston being careful not to touch the Teflon part,
- Screw the syringe on the filter,
- Pour the sample vial content (50 ml or less) into the syringe (which is now screwed to the filter),
- Write down the volume filtered,
- Insert the piston inside the syringe and filter all the sample until the filter grooves are visible once again. Stop pushing to avoid damage to the membrane.
- Make sure the reagent 2 MIN UNIT-ORAL® is at room temperature (~ 20°C) and put 4 drops of 2 MIN UNIT-ORAL® in the bottom of the plastic packaging that the filter came in,

From this stage, the following steps must be performed in a short space of time to obtain an optimal result.

- Place the filter tip in the bottom of the filter plastic packaging,
- Suck up all the reagent 2 MIN UNIT-ORAL® through the filter. Maintain the depression inside the syringe,
- By strong and constant pressure on the syringe piston, push the liquid out of the syringe into test tube until a thick foam comes out. Avoid an excess of foam in the test tube.
- Fix the test tube in the tube holder,
- Place them in the luminometer and press the ENTER button,
- Write down the R1 result in RLU (Relative Light Unit),
- Open the cover and get the test tube,
- Add one drop of STANDARD 1000. 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.
- Fix the test tube in the tube holder,
- Homogenize the mix,
- Place it in the luminometer and press the ENTER button,
- Write down the R2 result in RLU (Relative Light Unit),

5. Calculation of biomass quantity / Expression of results

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

**Calculation of the standard (in pgATP/RLU):**

\[
\text{STANDARD} = \frac{R2 - R1}{1000}
\]

**Calculation of the biomass value (in pgATP/ml):**

\[
[\text{ATP}] = \frac{R1}{\text{ETALON} \times V}
\]

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/pg ATP
- **V** = volume filtered in millilitres

It is possible to convert the ATP concentration (in pgATP/ml) to equivalent bacteria per millilitre (eq. bact./ml) by using the following rule: 1 picogramme ATP ≈ 1 000 bacteria. An Excel file is supplied by INSTITUT CLINIDENT to automatically perform the calculations presented above. The file can be used by filling the table with: date or location of the sampling, volume filtered and R1 and R2 values.
The Excel file gives the total biomass concentration for each sample in picogram ATP per millilitre, equivalent bacteria per millilitre, and the logarithm of equivalent bacteria per millilitre.

Dental Unit waterline sanitation is critical for patient safety. A symposium held at Trinity College, Dublin, Ireland, in Sept 2006 reached the consensus that output water quality from dental chair unit should comply with ADA standard (<200 cfu/ml).

- In Germany, it is recommended that only drinking water quality may be used in dental chair unit.
- In Europe, drinking water quality is < 200 CFU/ml.
- In USA/Australia drinking water quality is < 500 CFU/ml
- In Japan, drinking water quality is < 100 CFU/ml
- In France, water for any medical application should be < 200 CFU/ml in 2 minutes Unit-Oral chairside water quality gives quantitative results in equivalent CFU/ml with 4 levels of threshold represented by following colors:

- >500 CFU/ml
- 200< CFU/ml <500
- 100< CFU/ml <200
- <100 CFU/ml

2 minutes Unit-Oral is without any value for predicting whether the water is contaminated with potentially human pathogenic bacteria (E.coli, Entecoccus spp, Pseudomonas aeruginosa, Legionella pneumophila). These bacteria are analysed by q-PCR with MIC q-PCR instrument and GenoSPYD q-PCR reagents from stabilized dental unit water for shipment at room temperature in less than 10 days.

### Data

In the Excel file, for biomonitoring over time, there is one sheet per sampling point. Each line corresponds to one day of measurement. For cartography, there is only one sheet and each line
corresponds to a point of the network to control. If you want to perform several cartographies, you have to duplicate the sheet or replace the previous values.

Only the four grey columns must be completed. The other columns and graphs are filled in automatically.

The biomass concentration results are given in 4 different units. By default, we use the result in logarithm equivalent bacteria per millilitre. However, you can use one of the other three units. The results appear in different colours depending on the level of contamination. If it appears on a green background, no corrective action is necessary. If it appears on an orange background (between the warning threshold and the control threshold), there is no immediate danger. However, a corrective action is recommended if three consecutive results appears in orange. If the result appears on a red background, there is a high risk of microbiological development and a quick corrective action is recommended.

By default, this file is created with the theoretical thresholds corresponding to the type of water you are working on. Ideally, these thresholds should be adjusted to perfectly fit with the installation monitored.

Measurement anomaly

During measurement, it is possible that the standardisation is not correct. The Excel file will automatically warn you in case this anomaly is detected after filling the grey columns. Immediately get the test tube out of the luminometer, homogenise the mix and restart the measurement. If the problem continues, several possibilities should be considered:

- There is too much foam in the upper part of the tube that prevent the STANDARD 1000 from mixing with the sample. Restart the complete protocol avoiding an excess of foam and making sure the tube is correctly homogenized.
- The reagent 2 MIN UNIT-ORAL © is too cold. Warm up the tube to 20°C-25°C.
- The reagent 2 MIN UNIT-ORAL © is no longer active (out-of-date or degraded). Restart the complete protocol using a new bottle of 2 MIN UNIT-ORAL ©.
- The sample analysed has an inhibitory effect on the enzyme activity of the reagent 2 MIN UNIT-ORAL ©. Restart the complete protocol filtrating a smaller volume of water and rinsing the filter with sterile water.
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 1 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 reagents contamination

a) Test:
- In a test tube, put 2 drops of 2 MIN UNIT-ORAL®,
- 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 5 RLU.

b) Protocol to be followed in case of contamination:

Discard the contaminated reagent and select a new bottle of 2 MIN UNIT-ORAL®.

Control of the reagents efficiency

a) Test:
- In a test tube, put 2 drops of 2 MIN UNIT-ORAL® and 1 drop of STANDARD 1000,
- Homogenise the tube,
- Fix an empty test tube to the tube holder,
- Place it in the luminometer and press the ENTER button,
- The result should higher than 200 RLU.

b) Protocol to be followed in case of an efficiency loss:

Discard the contaminated reagent and select a new bottle of 2 MIN UNIT-ORAL®.