GENERAL INFORMATION

2 MIN PERIO/ENDO-ANALYSE ®

OPERATING MODE FOR QUANTIFICATION OF TOTAL FLORA IN PERIODONTAL POCKET OR IN ROOT CANAL BY ATP-METRY

- LUMINOMETER KIKKOMAN SMART -

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1. INSTITUT CLINIDENT overview

Founded in July 2003, INSTITUT CLINIDENT is a company 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 & Manufactures** 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 are from various sectors such as private clinics, hospital, dental school, university, oral care industry, research institutes.

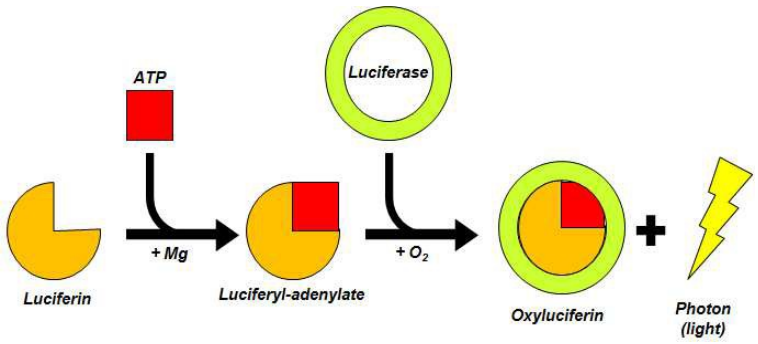
2. What is ATP-metry?

"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 biological sample, 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 (**2 minutes**).

3. Why use ATP-metry for microbiological monitoring?

The origin of periodontal disease is the formation of bacterial biofilm with specific pathogenic bacteria. The pathogenic bacteria are also present in the saliva of patients. The colonization of the mouth by periodontal bacteria can take place at an early age of the patient but can also take place after dental implant installation (peri-implantitis) if the patient has not been properly treated against pathogenic bacteria or having poor oral hygiene. It is important in daily clinical practice to evaluate the effect of the treatment by reduction of the bacterial loads to cure periodontal disease and prevent peri-implantitis.

During endodontic treatment, persistence of pathogenic bacteria strain in the root canal before closing, will increase the risk of new and rapid infection. Monitoring this risk is the key of endodontic treatment success.

The success of the long-term treatment is assured only if the patient practices a good oral hygiene and follows a monitoring program organized by dentists, with regular controls of the reduction of the bacterial loads into the periodontal pocket or absence after endodontic treatment.

With **PERIO-ANALYSE®** PCR (Polymerase Chain Reaction) kits, you are already able to **quantify** pathogenic bacteria and decide for specific treatment and re-evaluate after 3 months. This analysis is very powerful, but needs a specific laboratory to be performed, with a delay of several days.

With **2 MIN PERIO-ANALYSE®** you will be able, with a chairside solution, to monitor the efficiency of your treatment (bacterial load reduction) in 2 minutes.

- Before and after radicular surfacing and under-gingival descaling
- Before implant installation and during healing
- During bone augmentation procedure
- Before and after any surgical procedure
- At any time during maintenance protocol to evaluate biofilm reduction

The monitoring indicator should be a **rapid**, **reliable**, **easy to use** and **economic technology**. Quantitative ATP-metry is one of the best rapid indicators for biological monitoring of total bacterial load. Using **2 MIN PERIO-ANALYSE®**, you will:

- **Access to a validated method** to measure bacterial loads reduction,
- **Assess operating procedure efficiency:** validation of your clinical protocol (cleaning, disinfection ...),
- **Identify the critical point of your protocol:** determining critical points and highlighting malfunctions of your clinical procedure and therapeutic decision,
- **Motivate** the patient.

4. Protocol for quantification of total flora in periodontal pocket or in root canal

Before starting the protocol for quantification of total flora, please download the application on your smartphone.

https://app.institut-clinident.com/2min

a. Reagents: 2 MIN Starter Kit®

- 4 aluminium blisters containing 3 test tubes with lyophilised 2 min substrate
- 4 dropper bottles of **2 MIN Extractant**

Store test tubes and bottles of **2 MIN Extractant** at room temperature.

- 1 dropper bottle of **STANDARD 1000**

Store the bottle of **STANDARD 1000** between 2°C and 8°C.

b. Consumables: 2 MIN PERIO/ENDO-ANALYSE® Accessories Kit

- 12 absorbent paper points blisters (5 per blister), sealed and sterilised
- 12 vials of 5 ml of physiological saline solution
- 12 microtubes (for sample transfert – endodontic procedure only)

Store all accessories at room temperature.

c. Equipment needed

- 1 luminometer SMART
- **2 MIN CLINIDENT APP**

d. Procedure for Perio-Analyse : quantification of total flora in periodontal pocket

Phase 1: Installation

- On a flat and clean surface, prepare the luminometer, a dropper bottle of **2 MIN Extractant,** a dropper bottle of **STANDARD 1000**, a test tube with the lyophilised substrate, a vial of physiological saline solution.
- Turn on the luminometer (measurement chamber closed, empty) and wait 10 seconds for the device calibration.

Phase 2: Sampling in periodontal pocket

- Remove the supra-gingival dental plaque and isolate the sampling area with compresses or cotton rolls (to reduce contact with contaminated saliva from oral cavity).
- Open a vial of physiological saline solution.
- Take an absorbent paper point out of its package. Do not to touch the paper point with contaminated instruments (only use sterile devices and instruments) or fingers.
- Insert the paper point in the periodontal pocket by using tweezers. Leave in contact for 15 seconds and take out the paper point.
- Insert the paper point into the 5 ml vial of physiological saline solution.
- Repeat with other paper points until the periodontal pocket is dry. Put all the paper points in the same vial of physiological saline solution.

Phase 3: Quantification of the total flora in the sample

- Gently shake the vial of physiological saline solution during 30 seconds.
- Open an aluminium blister. Take a test tube with lyophilised 2 min substrate.
- Remove the cap of the test tube.
- Put 1 drop of physiological saline solution containing the paper points into the test tube.
- Put 4 drops of **2 MIN Extractant** into the test tube.
- Fix the test tube in the tube holder for SMART.
- Homogenize the mix by gently shaking.
- Place the tube holder in the luminometer. Close the cover and press the ENTER button.
- Write down the R1 result in RLU (Relative Light Unit).
- Open the cover and get off the test tube from the tube holder.
- Add one drop of **STANDARD 1000** into the test tube.
- Fix the test tube in the tube holder.
- Homogenize the mix.
- Place the tube holder in the luminometer. Close the cover and press the ENTER button.
- Write down the R2 result in RLU (Relative Light Unit).

Phase 4: Calculation of bacteria quantity / Expression of results in equivalent bacteria CFU

It is possible to convert the ATP concentration (in pg ATP) to equivalent bacteria CFU (Colony Forming Unit) by using the smartphone application supplied by INSTITUT CLINIDENT to automatically perform the calculations.

- Open the application on your smartphone. Select the Perio-Analyse icon.
- Enter the R1 result.
- Enter the R2 result.
- Result displays on your smartphone in **equivalent bacteria CFU**.

The analytical range of **2 MIN PERIO-ANALYSE®** is from 10^6 to 10^9 CFU per analysed sample.

2 MIN PERIO-ANALYSE® chairside smartphone application, gives a precise quantitative result in equivalent CFU with 4 levels of threshold represented by following colors:

$>10^9$ CFU
$10^8<CFU<10^9$
$10^7<CFU<10^8$
$<10^7$ CFU

2 MIN PERIO-ANALYSE® is without any value for predicting whether the periodontal pocket is contaminated with potentially periodontal pathogenic bacteria (*Aa, Porphyromonas gingivalis, Candida albicans*…). These pathogens are analysed by q-PCR with **MIC q-PCR instrument** and **PERIO- ANALYSE q-PCR reagents** from paper points after shipment to specialized laboratory to measure specific microbial reduction load.

e. Procedure for Endo-Analyse: quantification of total flora in root canal

Phase 1: Installation

- On a flat and clean surface, prepare the luminometer, a dropper bottle of **2 MIN Extractant,** a dropper bottle of **STANDARD 1000**, a test tube with the lyophilised substrate, a vial of physiological saline solution and a microtube (microtube is necessary only if the measurement is not performed immediately).

- Turn on the luminometer (measurement chamber closed, empty) and wait 10 seconds for the device calibration.

Phase 2: Sampling in root canal

- At the end of your root canal treatment, remove the supra-gingival dental plaque location and isolate the sampling area with compresses or cotton rolls (to reduce contact with contaminated saliva from oral cavity).

- After root canal decontamination with usual protocol, perform a quick rinse with physiological serum follow by a quick drying.

- Take an absorbent paper point out of its package. Do not to touch the paper point with contaminated instruments (only use sterile devices and instruments) or fingers. Insert the paper point in the root canal by using tweezers. Leave in contact for 10 seconds and take out the paper point.

  If you can not perform immediately the measurement, use the microtube to preserve the paper point until you move on to the next step. Repeat sampling with other paper points until the root canal is totally dry. Put all paper points in the same microtube.

If you can perform immediately the measurement:

- Open an aluminium blister. Take a test tube with lyophilised 2 min substrate.
- Remove the cap of the test tube (that contains the lyophilised enzyme).
- Insert the paper point directly into the test tube.
- Repeat sampling with other paper points until the root canal is totally dry.

Phase 3: Quantification of the total flora in the sample

- Open a vial of physiological saline solution.
- Put 10 drops of physiological saline solution into the test tube containing the paper points.
- Gently shake the test tube during 30 seconds.
- Put 4 drops of **2 MIN Extractant** into the test tube.
- Fix the test tube in the tube holder for SMART.
- Homogenize the mix by gently shaking.
- Place the tube holder in the luminometer. Close the cover and press the ENTER button.
- Write down the R1 result in RLU (Relative Light Unit).
- Open the cover and get off the test tube from the tube holder.
- Add one drop of **STANDARD 1000** into the test tube.
- Fix the test tube in the tube holder.
- Homogenize the mix.
- Place the tube holder in the luminometer. Close the cover and press the ENTER button.
- Write down the R2 result in RLU (Relative Light Unit).

Phase 4: Calculation of bacteria quantity / Expression of results

It is possible to convert the ATP concentration (in pg ATP) to equivalent bacteria CFU (Colony Forming Unit) by using the smartphone application supplied by INSTITUT CLINIDENT to automatically perform the calculations.

- Open the application on your smartphone. Select the Endo-Analyse icon.
- Enter the R1 result.
- Enter the R2 result.
- Result displays on your smartphone in **equivalent bacteria CFU**.

The analytical range of **2 MIN ENDO-ANALYSE ®** is from 10^4 to more than 10^6 CFU per analysed sample.

2 MIN ENDO-ANALYSE® chairside smartphone application, gives a precise quantitative result in equivalent CFU with 4 levels of threshold represented by following colors:

$>10^6$ CFU
$10^5<CFU<10^6$
$10^4<CFU<10^5$
$<10^4$ CFU

2 MIN ENDO-ANALYSE® is without any value for predicting whether the root canal is contaminated with potentially periodontal pathogenic bacteria (*Aa, Porphyromonas gingivalis, Candida albicans*…).

5. Anomalies, Controls, Information

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 prevents 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 Extractant** is no longer active (out-of-date or degraded). Restart the complete protocol using a new bottle of **2 MIN Extractant**.

- The sample analysed has an inhibitory effect on the enzyme activity of the reagent. Restart the complete protocol by diluting the sample (10 fold).

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) In case of contamination, follow this procedure:

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 Extractant**,
- 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) In case of contamination, follow this procedure:

Discard the contaminated reagent and select a new bottle of **2 MIN Extractant**.

Control of the reagents efficiency

a) Test:

- In a test tube, put 2 drops of **2 MIN Extractant** 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) In case of an efficiency loss of RLU signal, follow this procedure:

Discard the reagent and select a new bottle of **2 MIN Extractant**

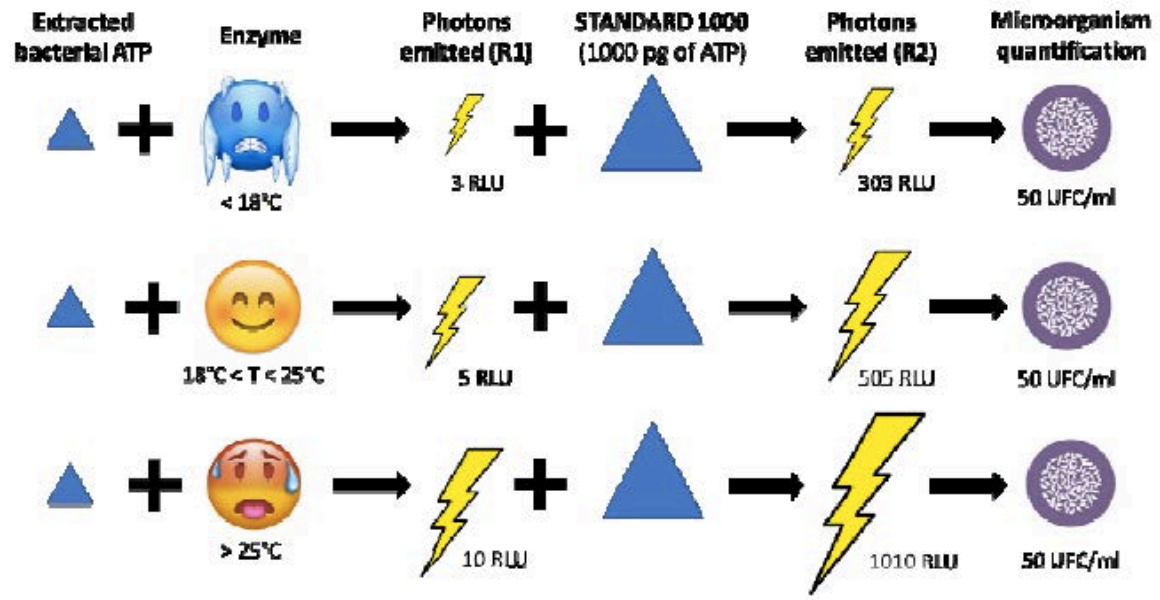
Informations: role of the internal calibration of ATP measurement

Bioluminescence analysis uses an enzyme that converts bacterial ATP into photons. Enzyme efficacy varies depending on environmental parameters such as temperature, pH, reagent aging... Consequently, the quantity of photons emitted varies for a same quantity of ATP.

The luminometer measures the quantity of photons emitted and displays the results in Relative Light Unit (RLU).

To take into account these parameters, each measurement is calibrated by adding one drop of **STANDARD 1000** which contains 1000 pg of ATP. This addition gives the correspondence between RLU and pg of ATP in the testing conditions following by correlation with equivalent CFU and quantitative results.

Example of the effect of temperature on photon emission:



R1 measurement variations are proportional to R2 measurement variations. With this standardization, the ATP technique is quantitative, reliable, robust, and measurements can be compared to each other. Internal calibration also acts as a quality control for each measurement.