INSTITUT CLINIDENT®
MY CHAIRSIDE LAB & PCR LAB

2 MIN PERIO/ENDO-ANALYSIS®: chairside evaluation of total microbial load in 2 minute

PCR PERIO-ANALYSIS ®: from 5 to 9 bacteria + *Candida albicans* in 48 Hours

MASTER PERIO-ANALYSIS®: PCR Perio-Analysis+ Mastocyte/Neutrophil Assay

- **Strengthen your diagnosis**
- **Optimize your protocol**
- **Reduce infectious risk**
- **Prevent recurrence**
- **Motivate your patients**

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PERIODONTAL PATHOGEN IDENTITY, QUANTITY AND ASSOCIATED RISKS

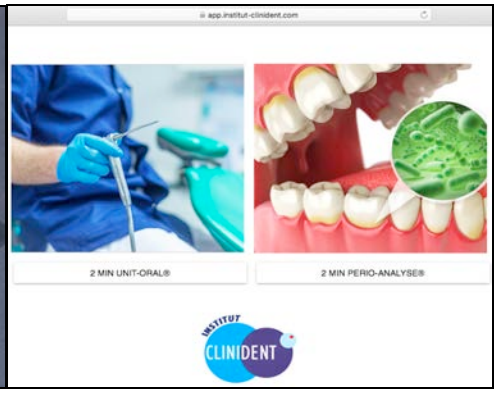
The origin of periodontal disease is the formation of bacterial biofilm with specific pathogenic bacteria. Pathogenic bacteria are also present in the saliva of patients. The colonization of the mouth by periodontal bacteria can take place at an early age of the patient but can also take place after dental implant installation (peri-implantitis) if the patient has not been properly treated against pathogenic bacteria or have poor oral hygiene. Microbial colonization associated with peri-implantitis is similar to bacteria identified during periodontal disease however high level of some bacteria seems more associated with peri-implantitis.

- ✓ **The non-existence in the oral cavity of a certain pathogenic bacteria strain will guarantee the absence of periodontal disease or peri-implantitis.**
- ✓ **The presence of certain pathogenic bacteria strains and fungus at a certain level will increase the risk of periodontal disease or peri-implantitis.**
- ✓ **Low periodontal pocket bacterial count reduces the risk for gum disease.**

2 MIN PERIO/ENDO-ANALYSE®: Chairside total count bacteria

The success of the long-term treatment is assured only if the patient practices good oral hygiene and follows a monitoring program organized by dentists and hygienists with regular Perio-Analyse controls.
The objective is not an absence but an acceptable level of the biofilm (if possible, less than 10 million per site) not leading to an inflammatory reaction, gum disease and destruction of the bone supports of the tooth. The effectiveness of a therapeutic protocol can be evaluated by measuring the reduction in the total microbiological burden.
Our handy lab **2 MIN PERIO/ENDO-ANALYSE®** measure Adenosine TriPhosphate (ATP) and evaluate total microbial count in 2 minutes. Reagents are dry and easy to manipulate.

2 MIN PERIO/ENDO-ANALYSE® KIT AND EQUIPMENTS



2 MIN PERIO-ANALYSE® REAGENTS AND READER

ATP concentration in Relative Light Unit (RLU) is converted into equivalent bacteria CFU (colony forming unit) by **2 MIN CLINIDENT-ANALYSE** application. The analytical range of **2 MIN PERIO-ANALYSE®** is from 10^6 to 10^9 CFU.

2 MIN PERIO-ANALYSE® smartphone application (APP) gives results 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 CLINIDENT® AVAILABLE KITS

	Vial with lysis buffer	Substrat buffer	Standard 1000	Physiologic Serum	Paper Points	Syringue + filters	Inter-dental brush	Surface swab
2 MIN PERIO-ANALYSE	•	•	•	•	•		•	•
2 MIN ENDO-ANALYSE	•	•	•	•	•			
2 MIN UNIT-ORAL	•	•	•			•		•

PCR PERIO-ANALYSE ®: 9 bacteria+*Candida albicans*+ Total bacteria count

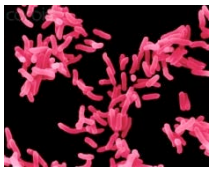
The presence of certain periodontopathogenic bacteria and some fungi increases the risk of periodontitis or peri-implantitis and may require antibiotic therapy. The choice of treatment depends on the bacterial load and the the composition of the subgingival flora.
List of pathogenic bacteria strains and fungus associated with risk of periodontal disease or peri-implantitis:

Detected Periodontal Pathogens	Basic Test	Basic + Test	Premium Test	Platinium Test
Aggregatibacter actinomycetemcomitans (Aa)	•	•	•	•
Porphyromonasgingivalis (Pg)	•	•	•	•
Tannerellaforsythensis (Tf)	•	•	•	•
Treponema denticola (Td)	•	•	•	•
Prevotella intermedia (Pi)	•	•	•	•
Peptostreptococcus micros (Pm)			•	•
Fusobacterium nucleatum (Fn)			•	•
Campylobacter rectus (Cr)			•	•
Eikenellacorrodens (Ec)			•	•
Candida Albicans (Ca)		•		•
Total Bacteria Count (TBC)	•	•	•	•

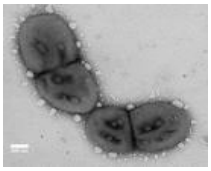
Periodontal diseases are generally Gram-negative which include *Actinobacillus actinomycetemcomitans, Tannerella forsythia* (previously designated *Bacteroides forsythus*), *Prevotella, Fusobacterium*, and *P. gingivalis,* (Socransky complex).Destructive tissue events requires a concerted interaction of these bacteria members (Marcotte and Lavoie, 1998; Maiden et al., 2003; Paster et al., 2006). Interdental microbiota in healthy population (aged 18–35 years-old) shows aggressive Socransky complex bacteria (Carrouel et al., 2016).
P. gingivalis mixed with other oral pathogens and deficiency of immunological factors in the host is essential for etiology of advanced periodontitis (Haffajee and Socransky, 1994). The number of *P. gingivalis* has been shown to increase substantially in sites with periodontitis and lower or non-detectable in sites with subgingival health or plaque-associated gingivitis (Schmidt et al., 2014).

A.a. and *P.g.* colonizes the gingival sulcus, invades through the epithelium and penetrates into the subgingival connective tissue to replicate in which ultimately results in catabolic alveolar bone loss (Bethany, 2016 ;Park, 2017.In presence of bacteria, immune response contribute to mast cell growing, degranulation and collagenase (MMPs) production with bone loss consequences (*Huang , 2013 ; .Malcolm, 2016).*Bone loss depend of the bacterial identity (including genotype for Aa and Pg) , bacteria quantity and host immune response *(Haffajee, 1994 ; Amano, 2003 ;Shimoyama, 2017)* .

Actinobacillus actinomycetemcomitans



Porphyromonas gingivalis



*S*urgical treatment or minimally invasive treatment did not statistically noticeably affect any disease-associated bacteria (Hagenfeld, 2018). Use of antibiotics resulted in a greater influence on the microbiome 3 months after therapy, but this difference disappeared at 6 months (Bizzaro, 2016; Mdala, 2013). A multicenter prospective cohort study analyzed 18,834 sites distributed on 3,139 teeth in 124 patients (data collected 5 times over a 24-month follow-up period) (Nomura, 2017). The salivary levels *Aggregatibacter actinomycetemcomitans and Porphyromonas gingivalis* were affected by clinical attachment level (CAL) progression. P. gingivalis counts of subgingival plaque from the deepest pockets is significantly associated with the progression of periodontitis (Kakuta , 2017).

Lower diversity (high % of red complex bacteria / total bacterial count) could indicate the presence of a stable and pathogenic biofilm, which is more difficult to eradicate even with the use of antibiotics due to the presence of some dominant (pathogenic) bacterial species (Mdala, 2013).

These results demonstrate the predictive value of specific subgingival bacterial profiles for the progression of periodontis, the support for decision to prescribe antibiotics and the importance of monitoring risk predictors during treatment and maintenance. This is the most important for success in periodontal treatment and reduction of associated chronic diseases (cardiovascular disease, artherosclerosis disease…).
A great number of periodontal disease cases can be treated and maintained under control for many years by dental hygien & protocols according to the traditional process of surgical mechanical actions, such as radicular surfacing and under-gingival descaling associated with hygiene protocol and some antibiotics. Choice of the therapy depends on the composition of the sub-gingival microflora. **An antibiotic therapy** can be prescribed only in combination with a meticulous cleaning of the pockets by surgery. Certain aggressive, anaerobic pathogenic bacteria are inside the soft tissue (intracellular) and require **surgical procedure** to eliminate contaminated soft and hard tissue.
The most aggressive periodontal pathogens, resulting in a high risk of rapid bone destruction and requiring specialized treatment are:

- *Aggregatibacter actinomycetemcomitans* (A.a): is almost insensitive to metronidazole but is sensitive to quinolone and tetracycline. Bone loss can reach more than 3 mm in 2 months with only 10^5 bacteria in the periodontal pockets (Haffajee 1994). He has a strong involvement in juvenile periodontitis. Antibiotics are always needed because it is an intracellular microorganism.
- *Porphyromonas gingivalis* (P.g): is sensitive to tetracycline and penicillin. The broad spectrum of metronidazole is not necessary. P.g is transmissible between parents and children as well as between partners. Bone loss can reach more than 2 mm in 2 months with only 10^5 bacteria in the periodontal pockets (Haffajee 1994).
- *Tannerella forsythensis* (T.f): is sensitive to tetracycline and penicillin. The broad spectrum of metronidazole is not necessary. The risk of bone loss is long term (several years). Antibiotic therapy is not always necessary.
- *Treponema denticola* (T.d): is sensitive to tetracycline and penicillin. The broad spectrum of metronidazole is not necessary. The risk of bone loss is long term (several years). Antibiotic therapy is not always necessary.
- *Prevotella intermedia* (P.i): may be resistant to penicillin. P.i is sensitive to tetracycline and metronidazole. The risk of bone loss is long term (several years).

Other pathogenic bacteria (*Peptostreptococcus micros, Fusobacterium nucleatum, Campilobacter rectus, Eikenella corrodens*) when combined and at high quantity will contribute to immune response and gum disease.

Periodontal disease antibiotic therapy has to be prescribed only after microbiological analysis to identify and quantify the exact bacteria present in the sulcus and decide the efficient antibiotic, reduce antibiotic resistance and treatment failure.

Candida albicans (C.a): is a fungus, it was found in the periodontal pockets in 7.1 to 19.6% of patients with chronic periodontitis. Candida albicans has also been isolated from the periodontal pockets of HIV-positive and diabetic patients. It can also be associated with peri-implantites. Antibiotics are not effective against fungi, local treatments are then proposed: antifungals such as nyastine, amphotericin, miconazole or fluconazole.

The success of the long-term treatment is assured only if the patient practices good oral hygiene and follows a monitoring program organized by dentists and hygienists with regular Perio-Analyse controls.
Patient suffering from periodontal disease are at risk of new bacterial infection around the dental implant (called peri-implantitis). Peri-implantitis increase at 16% of the patients and around 6.6% of the implants.

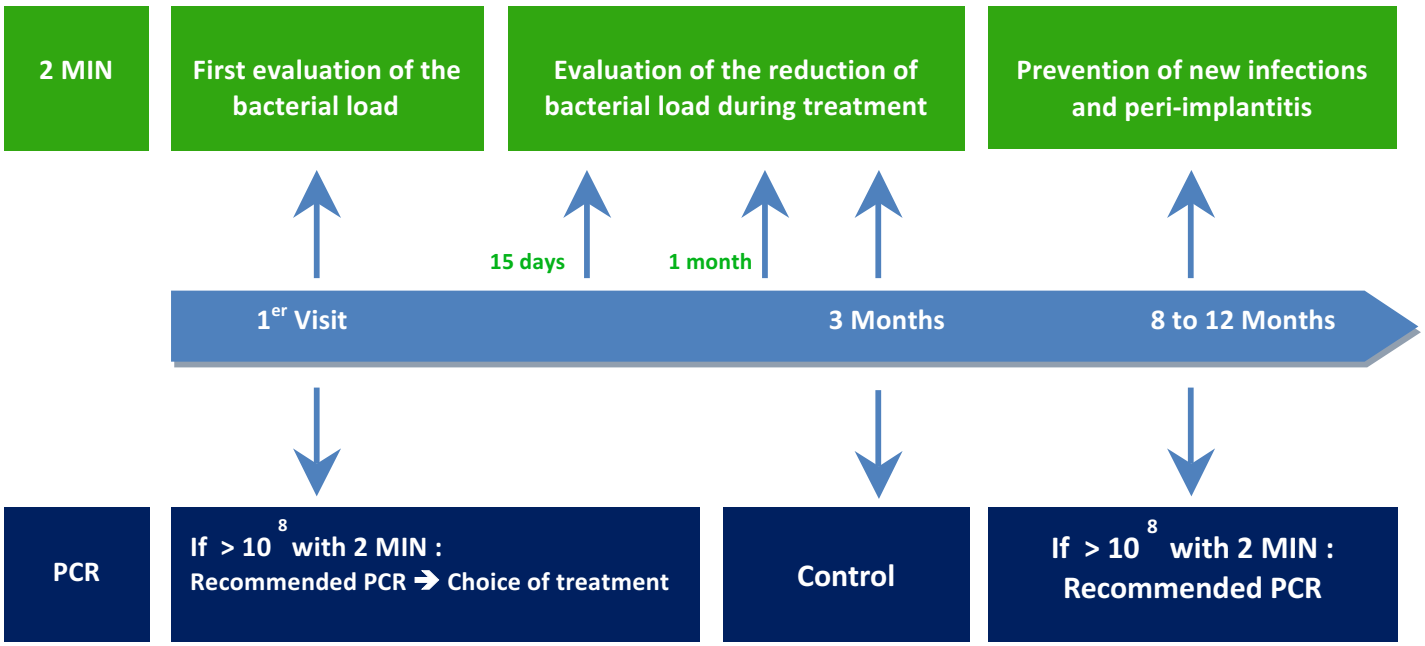
The microbial colonization associated with peri-implantitis is similar to bacteria identified during periodontal disease however high level of some bacteria seems more associated with peri-implantitis.

It is important in daily clinical practice to evaluate the biological risk before placing the implants, and supervising the patients after treatment to detect early signs of peri-implantitis infection. The early detection of signs of bacterial risk, the reinforcement of measurements of oral hygiene and the treatments will be able to reduce the bacterial loads and prevent peri-implantitis.

Used of 2 min Perio-Analyse and PCR Perio-Analyse is recommended to:

- **Strengthen your diagnosis**
- **Optimize your protocol**
- **Reduce infectious risk**
- **Prevent recurrence**
- **Motivate your patients**

2MIN+PCR PERIO-ANALYSE ®WHEN?



The objectives of the analysis:

- choice of a suitable antibiotic and treatment in relation with bacterial strain identity
- determine the quantity of bacteria in relation with pathogenic threshold
- follow-up of treatment and evaluation of success or failure
- early detection of the secondary infection
- motivation of patient to maintain their treatment and their oral hygiene in the long run

The PCR microbiological analysis gives a value of quantification (equivalent CFU by sample) of each periodontopathogen as well as the percentage of each bacterial type compared to the total flora.
It is advised to carry out an analysis in the following situations:

- first visit
- patients with periodontal disease risk or gingivitis
- smokers
- diabetic patients
- periodontal disease with depth of the pockets >4mm (in spite of a very good oral hygiene)
- aggressive or progressive periodontal disease
- refractory periodontal disease resistant to the therapy
- periodontal disease evolving/moving quickly
- peri-implantitis risk patient
- 3 months after periodontal treatment starting date
- every 6 months for patients with high risk
- every year for all patients.

MASTER PERIO-ANALYSIS®: PCR Perio-Analysis+ Mastocyte/Neutrophil Assay

Many studies describe the inflammatory response of the individual in the presence of periodontal germs, followed by the immune response to explain the destruction of periodontal tissues. Periodontal pathogens use inflammation to provide a favorable environment for growth. Therefore, in addition to measuring attachment loss and finding responsible pathogens, the focus should be on inflammatory biomarkers. The quantitative analysis in immuno-cytology of mast cells-neutrophils (the result of which is the overproduction of destructive enzymes such as collagenase / metalloproteinases MMPs), coupled with the microbiological analysis allows to better position the patient risk and evaluation of chronic inflammatory conditions.

Detected Periodontal Pathogens	Master Test
Aggregatibacter actinomycetemcomitans (Aa)	•
Porphyromonas gingivalis (Pg)	•
Tannerella forsythensis (Tf)	•
Treponema denticola (Td)	•
Prevotella intermedia (Pi)	•
Peptostreptococcus micros (Pm)	•
Fusobacterium nucleatum (Fn)	•
Campylobacter rectus (Cr)	•
Eikenellacorrodens (Ec)	•
Candida Albicans (Ca)	•
Total bacterial count (TBC)	•
Immunocytology (Inflammation Marker)	•

References: scientific publications could be provided to dentists under specific demand by email. Please contact info@institut-clinident.com or the institute clinident webpages (www.institut-clinident.com) and ask for publications.

FAQ

Why total bacteria count is important?

- The presence of a flora> 10^8 bacteria in a pocket indicates a non balanced flora.
- The lack of reduction of this flora following chair treatment indicates low treatment efficiency and the need to consolidate the diagnosis and the treatment protocol.

Why not only collect saliva?

- Because the presence of periodontal bacteria into the saliva is a risk factor, but not a diagnosis.

Which bacterias are at high risk for rapid bone loss?

- Aa and Pg.

Why Aa required strong surgical/mechanical treatment with flap?

- Aa is intracellular bacteria and could also survive in presence of oxygen after surgery.

Why Candida albicans could be a risk?

- Because in presence of antibiotic, Ca can grow and fully colonize the sulcus.

What are the unit used for bacteria load?

- 10^4 = E+04 = 10 000 bacteria in the tested sulcus.

What is PCR?

- PCR is amplification of specific zone of the DNA (Polymerase Chain Reaction)
- Each amplified zone is specific from a bacteria species
- PCR process takes about 6 hours.

When should I test my patient?

- For PCR, before treatment decision and 3 months after the first sampling and antibiotic use.
- For 2 MIN, 2 to 3 weeks after the end of initial chair treatment & compare with the initial test.

Antibiotic recommendation?

- Aa alone or with red complex = tetracycline
- Red complex > the threshold = penicillin
- Red complex + Pi > the threshold or Pi alone = metronidazole.

May I use probiotics?

- Yes, after perio treatment, probiotics will assure a microbial competition and reduce the risk of new infection.

How many paper points should I use?

- Minimum 2 per site. 10 paper points for a pool sample (for representativity).

How long is stable DNA on paper point at room temperature?

- > 2 weeks.

What at are the DNA target and DNA standard of the PCR developed by Institut Clinident?

- Ribosomal 16S DNA sequences
- Qualibrated DNA from DSMZ (Germany) and Institut Pasteur (France).

What is the full PCR bacteria list developed by Institut Clinident?

1. *Aggregatibacter actinomycetemcomitans*
2. *Porphyromonas gingivalis*
3. *Tannerella forsythia*
4. *Treponema denticola*
5. *Fusobacterium nucleatum*
6. *Prevotella intermedia*
7. *Prevotella nigrescens*
8. *Parvimonas micra*
9. *Campylobacter Gracilis*
10. *Campylobacter rectus/showae*
11. *Eubacterium nodatum*
12. *Eikenella corrodens*
13. *Capnocytophaga species*
14. *Campylobacter concisus*
15. *Streptococcus mitis*
16. *Streptococcus gordonii*
17. *Streptococcus constellatus*
18. *Actinomyces viscosus*
19. *Actinomyces odontolyticus*
20. *Veillonella parvula*
21. *Enterococcus faecalis*
22. *Streptococcus mutans*
23. *Lactobacillus spp.*
24. *Candida albicans*