ORALDNA® LABS
Innovations in Salivary Diagnostics

MyPerioPath®

December 2016 Test Updates and Enhancements

We sincerely appreciate the thousands of dental and medical offices that use MyPerioPath® for early warning of oral pathogens, personalization of periodontal treatment and identification of related systemic risks. We are excited to announce that this essential test is getting a major upgrade as of Monday, December 5, 2016. (Not yet available in NY)

So, what is new? ...
New Technology. We have made large investments in the latest laboratory automation and chemistry technologies, which will provide more sensitivity, accuracy, and reproducibility for all pathogens tested.

More Targeted Sub-Types & Strains. The 11 reported pathogens are the same, but certain bacteria (e.g. Fn and Td) are now more comprehensively evaluated to include pathogenic sub-types and strains, consistent with the latest clinical research.

Updated Therapeutic Thresholds. The Therapeutic Thresholds (a.k.a. the black lines across the colored bars in the report graphic) have been updated to reflect correlations with clinical data reported to us from a large data set of patient samples with periodontitis.

This White Paper provides detail on the changes and methodology.

New Technology for MyPerioPath®

Several major innovations in molecular diagnostics technology have been incorporated into MyPerioPath® to improve its performance.

Real-time quantitative polymerase chain reaction (qPCR) is quickly becoming the industry standard and OralDNA® Labs has made the investment into cutting-edge instrumentation that incorporates this technology. qPCR measures patient bacterial DNA amounts continuously throughout the processing of each test. The results of the patient samples are compared against a series of known controls tested using the same process. The controls are then used to accurately calculate the amount of bacterial DNA in the patient sample.

The upgraded laboratory equipment also provides increased sensitivity, reflected in a wider dynamic range of bacteria that can be detected and accurately quantified. The enhanced MyPerioPath® achieves a 5 orders of magnitude dynamic range from less than 500 bacteria to more than 1 million. Additionally we have shown greater precision (i.e. reproducibility) increasing confidence when evaluating patient response to personalized therapy.

Another key innovation is the universal application of a Saliva DNA Preservative which prevents bacteria, yeast and virus growth after collection to ensure an accurate report. The preservative has been evaluated with MyPerioPath® for more than a year and the test enhancements have now been optimized for the preservative.

Updated Targeted Sub-Types & Strains

Based on the latest research we have redesigned the reaction components needed to detect the pathogenic bacteria *Treponema denticola* (Td) and *Fusobacterium nucleatum* (Fn). We now know there are more types or sub-species of these organisms that play a part in disease[1,2].

The Td and Fn design now detects several newly characterized pathogenic sub-species. *Fusobacterium nucleatum* will include the subspecies polymorphum, nucleatum, vincentii, and animalis[1,2]. The enhanced detection of Fusobacterium species is clinically important as this group of bacteria works in concert with the red complex bacteria such as Aa, Pg, Td and Tf and are linked to complications of pregnancy and increased risk related to cardiovascular disease and diabetes[3,4].

Updated Therapeutic Thresholds

Background

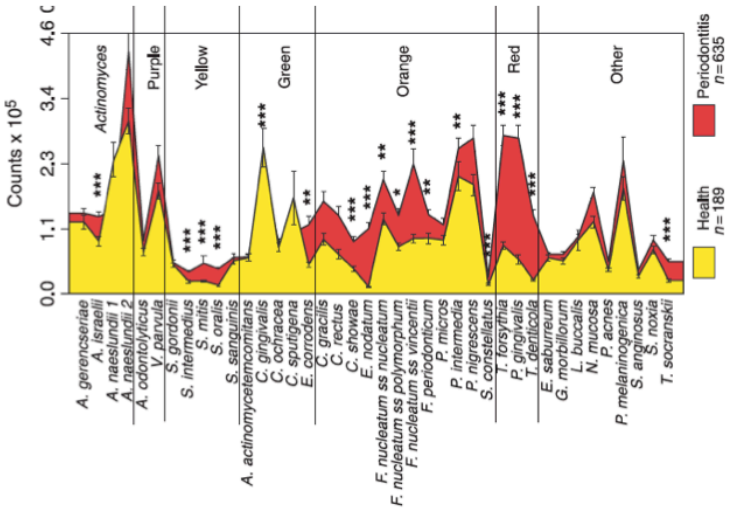
The causes of chronic periodontitis are multiple, including a variety of oral bacteria, viruses and fungi and the corresponding inflammation that is the response to these infections. Oral bacteria are ubiquitous, with more than 500 characterized species, some of which are pathogenic [5-7]. For the most part these pathogenic bacteria are part of the normal flora at low levels. However when levels of bacteria exceed the normal range, these bacteria are a key marker of the severity of periodontal disease, and indicate when a patient needs to be treated.

Socransky and colleagues are to be credited with the classic research leading to an understanding of bacteria that are pathogenic and what levels of those bacteria are considered to be clinically important[8-10].

A key study by Teles *et al*. studied oral bacteria in two groups of patients, one healthy and one with clinically diagnosed periodontitis[11]. Figure 1 depicts the quantitative range of each respective species of bacteria between the healthy (yellow) and periodontitis

(red) groups. The levels of the bacteria are highly variable, ranging over 5 fold. In most cases, the quantity of each bacterial species is greater in the patients with periodontitis, though the lower range and the variance of that range are widely disparate. Although the breadth of the severity of disease within the affected group of patients is not obvious from these studies, there is a clear conclusion that the amount of bacteria correlates with the presence and presumably the progression of disease. Historically, clinicians have referred to the calculation of bacterial levels as a basis of decisions of when and when not to treat patients.

Figure 1: Bacteria levels between healthy (yellow) and periodontitis (red) groups.



Greater precision in determining the relationship between bacterial levels and disease severity can be achieved when more specific clinical information is used. This clinical information may include commonly accepted systems to classify patients including the ADA criteria, the direct measurement of pocket depth and or the AAP system of patient classification. For samples collected and tested for OralDNA® Labs, this key clinical information is captured and used to make the correlations between bacterial load and disease severity.

Updated Approach

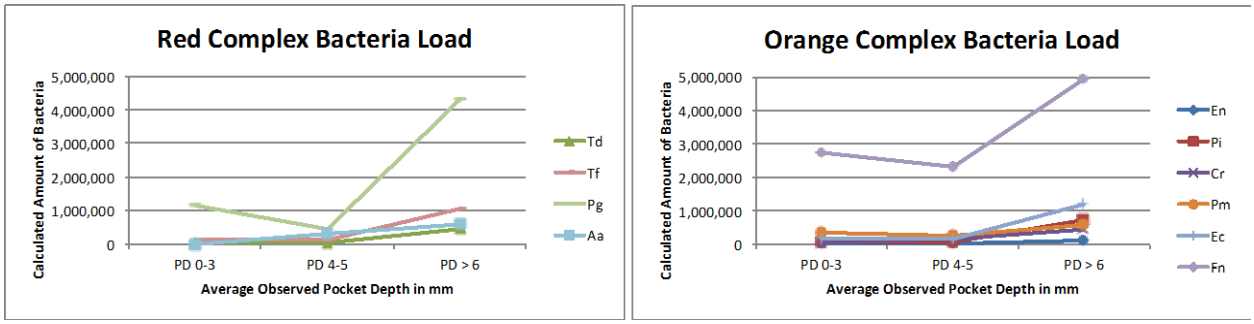
Therapeutic Thresholds have been updated in the updated version of MyPerioPath®. The *Therapeutic Threshold* is defined as the level of bacterial load, above which there is a high confidence of a clinically important infection, and the consensus to recommend the treatment of that infection.

Previously, OralDNA® Labs used thresholds derived from literature citations, in particular Teles et al[11]. Thresholds are now established from our own testing experience, correlated with clinical symptoms reported to us. The following section explains *Therapeutic Threshold* in more detail.

Important demographic and clinical information from a large cohort of patients was collected to better understand the pathology of periodontitis. This approach allowed us to validate Therapeutic Threshold levels from our own testing experience through correlation with clinical observations reported to us by providers.

We analyzed our data for a large number of independent variables, including signs of bleeding on probing, documentation of bone loss, ADA classification and intrinsic to that categorization, the mean and maximal measured pocket depth to determine which clinical variable was most correlated with disease severity. We found pocket depth to be most correlated with disease. Within the range of clinical findings, the level of pocket depth, and hence of periodontal disease severity where most clinicians agree to treat, is an average of 4-5 mm throughout the mouth[12,13]. Figure 2 illustrates the relationship of the amount of periodontal bacteria (bacterial load) to pocket depth using the updated version of MyPerioPath®. The graphs show an increasing amount of bacteria throughout, but in general, a marked increased in bacterial load beginning with a pocket depth 4-5 mm.

Figure 2: The relationship between pocket depth and the amount (bacterial load) of Red and Orange complex bacteria.

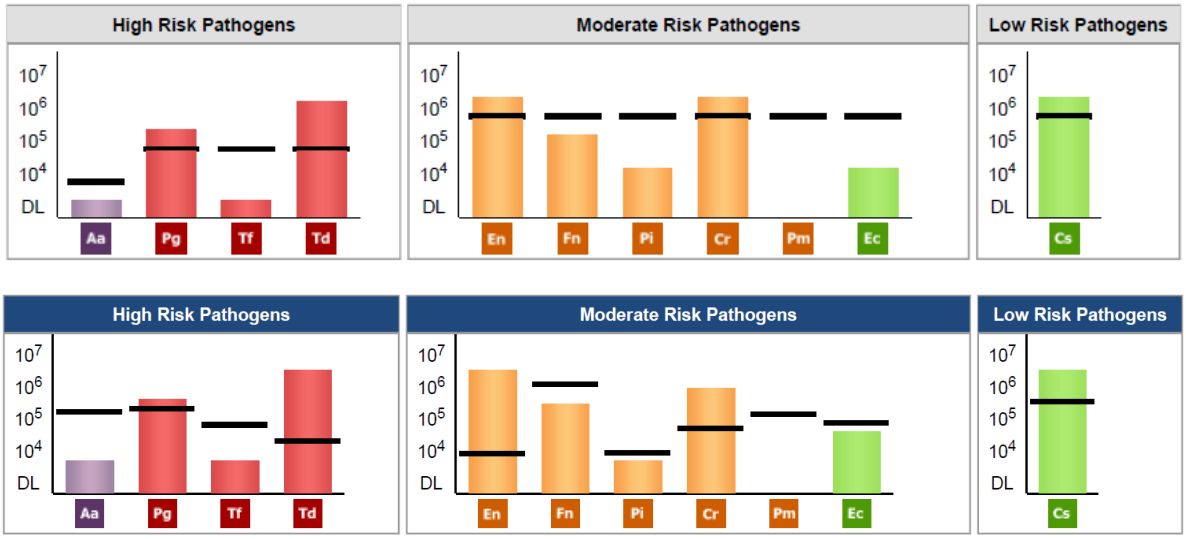


The goal of the *Therapeutic Threshold* is to provide a consensus reference of test results to be used in treatment planning. Accordingly, the definition of the therapeutic threshold, depicted as the black lines drawn in alignment with the histogram bars for each bacteria reported, is that level of bacteria that corresponded to a consensus, or unquestioned degree of disease severity so as to guide clinicians as to when they might always consider treatment for a patient without specifying a particular treatment modality.

Results

Figure 3 below shows the current and revised level of bacteria that comprise the Therapeutic Thresholds for the MyPerioPath®, in a logarithmic scale. For example, the current threshold of the bacteria Aa is 10,000 bacteria/milliliter (mL) of sample that is 10 exponent 4 or log 4.0. As is shown in the table, the range of threshold for the various bacteria is between log 4.0 to log 6.0. The updated thresholds show greater precision in the calculation.

Figure 3. The figure illustrates the current and updated levels of the *Therapeutic Threshold* for each of the assayed bacteria in the MyPerioPath® test. Upper panel the current MyPerioPath®, the lower panel in the updated version of the test.



As can be seen in Figure 3, the changes in the *Therapeutic Threshold* for the various bacteria assayed are similar to those in the current version of MyPerioPath®, but more precise. The new thresholds show an improved correlation between the calculated bacterial load and clinical measures of disease severity. The updated thresholds, in combination with increases accuracy and a larger dynamic range may lead to minimal differences in patient results between the new and current version of the test.

Below are the expected changes for the new MyPerioPath® for individual bacterial results, observed from our extensive validation work:

- En, Pi, Cs, Ec, and Pg will remain the same for incidence and above *Therapeutic Threshold* rates
- Td, Pm, Tf, and Fn will have slightly increased incidence and above *Therapeutic Threshold* rates
- Cr and Aa will have lower incidence but the same above *Therapeutic Threshold* rates

These values may serve to refine a clinician expectation as to the frequency of seeing these levels of bacteria in their patients.

What's Next?

With increased sensitivity and reproducibility, MyPerioPath® is expected to have a larger role in providing an early warning to patients at risk for periodontal disease. As such, younger patients with no obvious signs of inflammation would be benefited to know which bacteria are present in their mouth and which bacteria are at levels normally requiring treatment.

MyPerioPath® can also help in identifying increased risk of systemic disease related to periodontitis or specific pathogenic bacteria. Much has been studied and reported about atherosclerotic cardiovascular disease, birth complications inflammation of the pancreas associated with type II diabetes, and new evidence linking certain bacteria with the risk of pulmonary and pancreatic cancers[3,14-16].

Our plan is to add further information on systemic risk to the MyPerioPath® test report in the near future. We are working on expanded therapeutic considerations to guide clinicians in the emerging integration of dentistry and medicine.

References

1. Nie S, Tian B, Wang X, et al. Fusobacterium nucleatum subspecies identification by matrix-assisted laser desorption ionization-time of flight mass spectrometry. Journal of clinical microbiology 2015;53:1399-402.
2. Dzink JL, Sheenan MT, Socransky SS. Proposal of three subspecies of Fusobacterium nucleatum Knorr 1922: Fusobacterium nucleatum subsp. nucleatum subsp. nov., comb. nov.; Fusobacterium nucleatum subsp. polymorphum subsp. nov., nom. rev., comb. nov.; and Fusobacterium nucleatum subsp. vincentii subsp. nov., nom. rev., comb. nov. Int J Syst Bacteriol 1990;40:74-8.
3. Han YW, Houcken W, Loos BG, Schenkein HA, Tezal M. Periodontal disease, atherosclerosis, adverse pregnancy outcomes, and head-and-neck cancer. Adv Dent Res 2014;26:47-55.
4. Han YW. Fusobacterium nucleatum: a commensal-turned pathogen. Curr Opin Microbiol 2015;23:141-7.
5. Bizzarro S, Loos BG, Laine ML, Crielaard W, Zaura E. Subgingival microbiome in smokers and non-smokers in periodontitis: an exploratory study using traditional targeted techniques and a next-generation sequencing. Journal of clinical periodontology 2013;40:483-92.
6. Divaris K, Monda KL, North KE, et al. Genome-wide association study of periodontal pathogen colonization. Journal of dental research 2012;91:21S-8S.
7. Lourenco TG, Heller D, Silva-Boghossian CM, Cotton SL, Paster BJ, Colombo AP. Microbial signature profiles of periodontally healthy and diseased patients. Journal of clinical periodontology 2014;41:1027-36.
8. Haffajee AD, Cugini MA, Tanner A, et al. Subgingival microbiota in healthy, well-maintained elder and periodontitis subjects. Journal of clinical periodontology 1998;25:346-53.
9. Haffajee AD, Socransky SS. Microbiology of periodontal diseases: introduction. Periodontology 2000 2005;38:9-12.
10. Socransky SS, Haffajee AD. Dental biofilms: difficult therapeutic targets. Periodontology 2000 2002;28:12-55.
11. Teles RP, Haffajee AD, Socransky SS. Microbiological goals of periodontal therapy. Periodontology 2000 2006;42:180-218.

12. Slots J, Emrich LJ, Genco RJ, Rosling BG. Relationship between some subgingival bacteria and periodontal pocket depth and gain or loss of periodontal attachment after treatment of adult periodontitis. Journal of clinical periodontology 1985;12:540-52.
13. Kaye EK, Chen N, Cabral HJ, Vokonas P, Garcia RI. Metabolic Syndrome and Periodontal Disease Progression in Men. J Dent Res 2016;95:822-8.
14. Michaud DS. Role of bacterial infections in pancreatic cancer. Carcinogenesis 2013;34:2193-7.
15. Scannapieco FA, Cantos A. Oral inflammation and infection, and chronic medical diseases: implications for the elderly. Periodontol 2000 2016;72:153-75.
16. Zeng XT, Xia LY, Zhang YG, Li S, Leng WD, Kwong JS. Periodontal Disease and Incident Lung Cancer Risk: A Meta-Analysis of Cohort Studies. J Periodontol 2016:1-13.