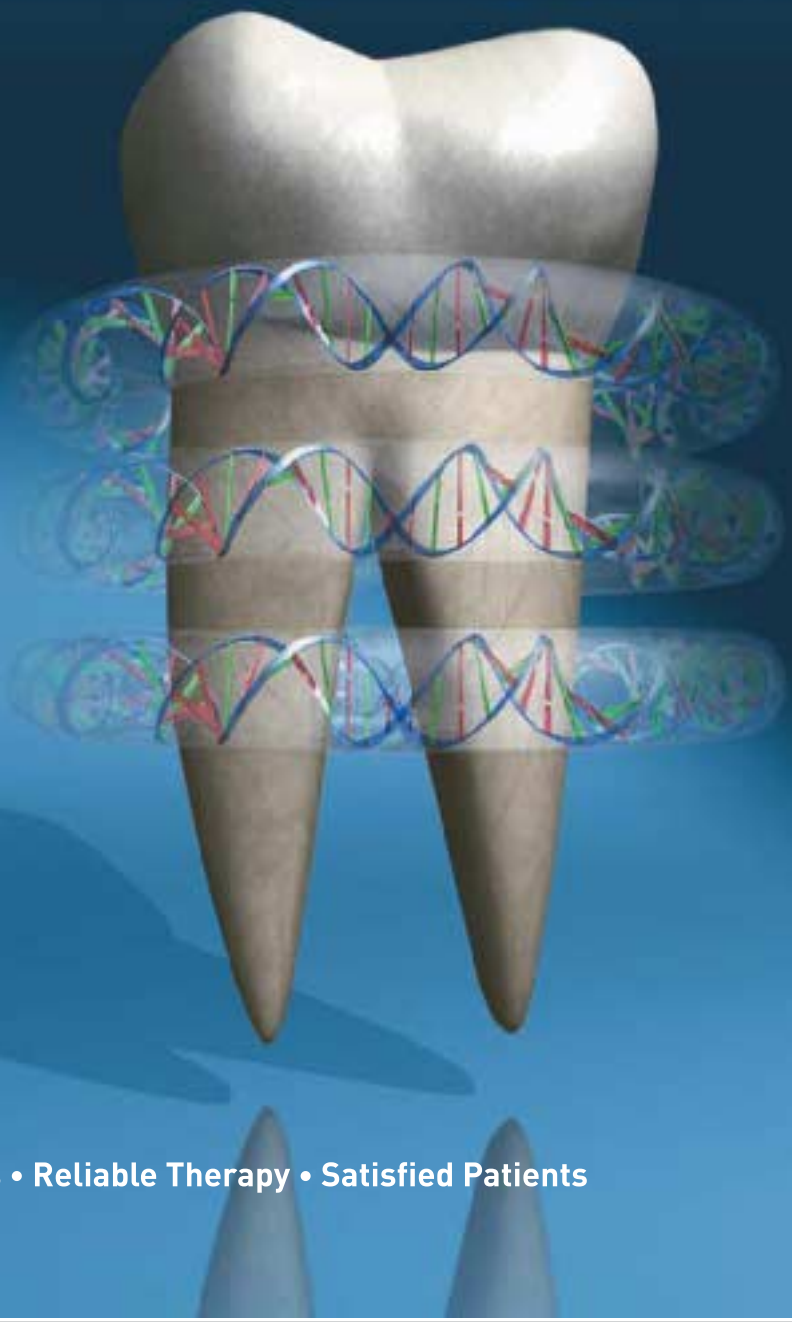
Periodontitis

Diagnostics and Therapy from Hain Lifescience



microDent®:
Marker Pathogen Analysis

GenoType®PST®:
Genetic Risk Evaluation

- CE Marked
- Verified Quality
- ISO 9001 Certified Quality

Sound Diagnosis • Reliable Therapy • Satisfied Patients

HAIN LIFESCIENCE

Info: www.hain-lifescience.de

Periodontitis as an Infectious Disease

It is now certain that a definite group of highly pathogenic bacteria is the primary cause of progressive periodontal diseases (PA diseases). This leads to the need for specific diagnostics and therapies of the causative bacteria especially in cases of chronic progressive, therapy-resistant and aggressive periodontal diseases.
Purely mechanical treatment will, by itself, normally not prove effective where these tissue-invasive periodontal pathogens are present in the oral cavity, but rather antibiotic methods must be employed. Knowledge of the bacterial spectrum is therefore a prerequisite for choosing an appropriate antibacterial agent.

Periodontal Diagnostics: Certified Marker Pathogen Analysis with microDent®

The **microDent®** test is an ISO certified molecular biological test for the analysis of periodontal pathogenics, and its diagnostic quality has been proven through extensive research studies carried out by university clinics (e.g. Eick & Pfister, J. Clin. Periodontol. 2002). The test is characterized by simple sampling and easy transport (no viable organisms required) as well as high diagnostic sensitivity and specifity. The **microDent®** test is based on the **DNA•STRIP®** technology developed by Hain Lifescience and is comprised of a highly sensitive and highly specific molecular biological PCR-DNA-probe method.

Periodontitis is Hereditary

The risk of developing periodontitis is genetically determined. Thus, where there is a genetic defect in the immune system, there will be an over-production of the main inflammatory mediator interleukin-1. This causes a fulminant reaction in the periodontal bone and connective tissue leading to a large degree of attachment loss.

Periodontitis Diagnostics: Risk Evaluation with GenoType® PST®

By taking a painless buccal swab, the **GenoType® PST®** can be used to determine the individual risk to develop a profound peridontitis or an implant loss. Patients with a positive interleukin genotype require an ongoing intensive therapy and prophylaxis plan in order to prevent loss of natural teeth or implants.
The **DNA•STRIP®** technology from Hain Lifescience is characterized by its high quality and safety and simple application within the routine laboratory environment. Further information to this method can be found on page 4 or ask for our free technique brochure.

Etiology of Periodontitis

In Germany the incidence of adult periodontitis needing treatment is approximately 75%. Patients, today, aged 40 experience a much greater degree of tooth loss as a result of periodontal diseases than that caused by caries. The chief causes of the development of periodontal disease are periodontal pathogens (PA bacteria), genetic predisposition within the immune system, poor oral hygiene, smoking, systemic diseases and stress (see Fig 1). Where an active periodontitis is developing, the body's defence system plays a central role. Normally low concentrations of periodontal pathogens present even in a healthy sulcus can be kept in check by an intact immune system.
However, if the defence system is impaired by a genetic predisposition (interleukin-1 polymorphism), medication or smoking, the bacteria can proliferate freely leading to the manifestation of profound periodontitis.

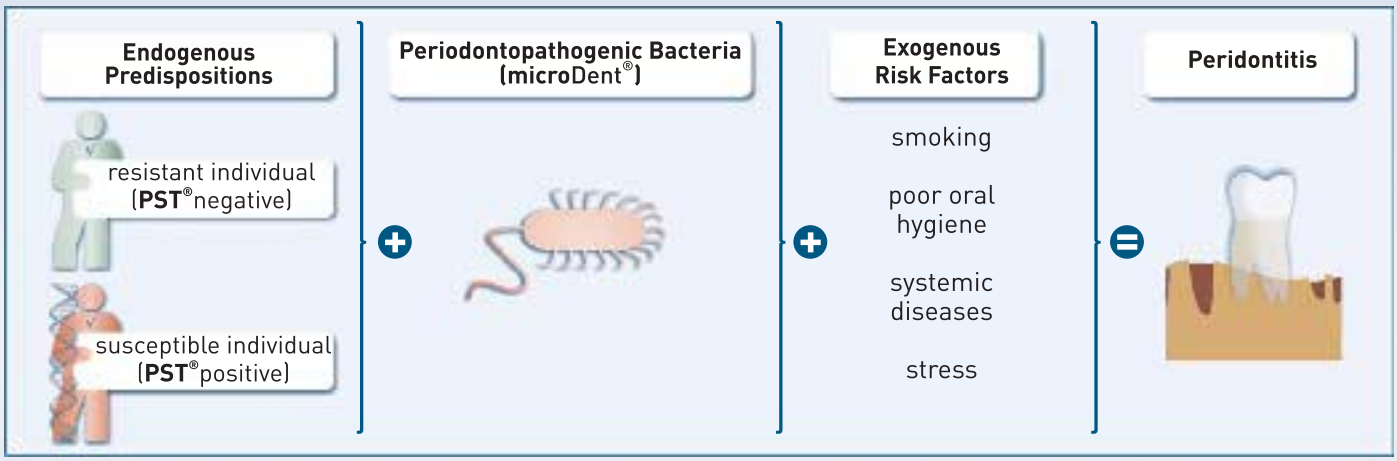


Fig 1

Periodontal Marker Bacteria and Pathogenicity

Studies by Slotz *et al.* (1997), Socransky *et al.* (1998, 2000) among others, have shown that only a few of the approximately 400 bacterial species present in the oral cavity have a high pathogenic potential that can cause profound periodontal disease (see Table 1). The marker pathogens of periodontitis belong to the group of obligatory anaerobic black-pigmented bacterial species such as *Actinobacillus actinomycetemcomitans* (synonym: *Heamophilus actinomycetemcomitans*), *Porphyromonas gingivalis, Bacteroides forsythus* (synonym: *Tannerella forsythensis*), *Prevotella intermedia, Treponema denticola* among others. The periodontitis-associated bacteria are characterized by the production of various metabolites together with virulence factor properties leading either to the direct destruction of the surrounding periodontal tissue or the inactivation of the humoral host defence. In particular, the highly pathogenic species *Actinobacillus actinomycetemcomitans, Porphyromonas gingivalis* and *Bacteroides forsythus* possess a whole range of pathogenic factors, and their presence in the gingival pocket has the potential to cause further tooth loss. In addition to these highly pathogenic species, other moderate pathogenic species may also have a pathogenic potential dependent upon the concentrations in which they are present.

very strongly pathogenic	strongly pathogenic	moderately pathogenic
Actinobacillus actinomycetemcomitans	Prevotella intermedia	Prevotella nigrescens
Porphyromonas gingivalis	Treponema denticola	Peptostreptococcus micros
Bacteroides forsythus		Fusobacterium nucleatum
		Campylobacter rectus
		Eikenella corrodens

Table 1

Molecular Genetic Diagnostics of Periodontal Marker Pathogens

Recent molecular genetic test assays represent the method of choice for the diagnosis of periodontal disease.
The **microDent®** test from Hain Lifescience sets new standards in the quality of diagnostics of periodontal bacteria by combining DNA amplification with subsequent detection using DNA probes.
Once the bacterial DNA has been isolated, it is multiplied (amplified) millionfold in a highly specific copying process. This process, known as polymerase chain reaction (PCR), takes place on the level of the nucleic acids and therefore does not require any viable organism. Due to the high specificity of the PCR, any potential contamination of the probe by concomitant flora has no influence on the test result (see Fig 2).
The amplified DNA is applied to the **DNA•STRIP®** matrix where specific probes are located. These probes hybridize in a specific and sensitive manner with the amplified nucleic acid derived from the specimen.
The reaction between the probe and the amplified DNA causes a color reaction on the **DNA•STRIP®**, the degree of which correlates with the amount of initial DNA in the probe (see Fig 3).

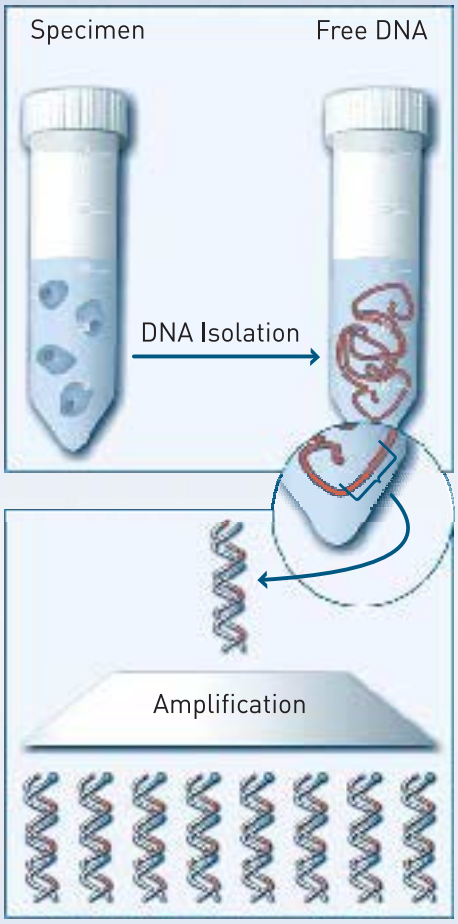


Fig 2

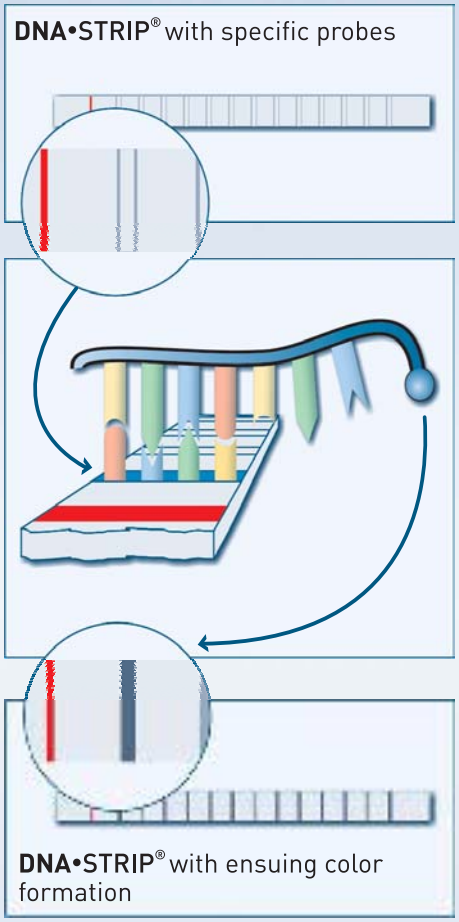


Fig 3

The combination of amplification and hybridization provides a much more dependable diagnosis in comparison with other methods. False positive and false negative results are practically impossible to reach.

The **GenoType® PST®** test for genetic risk evaluation is based on the same test principle.

Indications for the microDent® test

with
- PAs with pocket depth > 4 mm (despite excellent oral hygiene)
- therapy resistant, refractory adult periodontitis
- agressive and progressive PAs
- infections of osseointegrated implants

for
- choice of appropriate antibiotics
- monitoring of therapy success
- early diagnosis of recidives during recall
- estimation of the risk of implantation failure prior to an extensive prosthetic rehabilitation
- patient motivation for better compliance

Sound Diagnosis
↓
Reliable Therapy
↓
Satisfied Patients

Important Infomation about the microDent® Test

Test Range
The **microDent®** test enables the detection of the five most important periodontitis-associated marker bacteria: *Actinobacillus actinomycetemcomitans, Porphyromonas gingivalis, Bacteroides forsythensis, Prevotella intermedia* and *Treponema denticola.*
The **microDent®*plus*** version provides, moreover, detection capabilities for a further 6 periodontal bacteria with pathogenic potential.

Instructions for Taking a Specimen
The specimen is taken in the dental practice.The following points should be observed:
1. Prior to sampling, the supra-gingival plaque should be removed with a sterile curette and the site of sampling be dried with a sterile cotton roll.
2. Principally sampling should be done prior to mechanical treatment of the pocket. Whereas contamination with blood does not affect the test result, the sampling of severely inflamed pockets which are producing pus should be avoided.
3. Using a pair of sterile forceps insert one paper point at a time into the pre-defined sites down to the base of the sulcus. Leave the paper point in this position for 10 seconds.
4. Transfer the paper point to the respective transfer tube and record the site of sampling on the order form.

Test Variations
a) screening (see Fig 4)
Where it is only necessary to establish the presence of a pathogen rather than it's concentration or localization, e.g. as a means of assessing therapeutical success, a "multi-site sample" is recommended. In this case up to five paper points with swipes from single gingival pockets can be pooled in the transfer tube with the red screw cap.

b) semi-quantitative testing, single analysis (see Fig 5)
In order to obtain information about the bacterial flora of a single gingival pocket, the sampling may be carried out with one paper point per sulcus. Up to four analyses per patient can be requested with any one order. The transfer tubes are color-coded to avoid mixing of specific samples in the dentist's practice or the laboratory. The assignment of a color to a sample should be recorded on the order form.

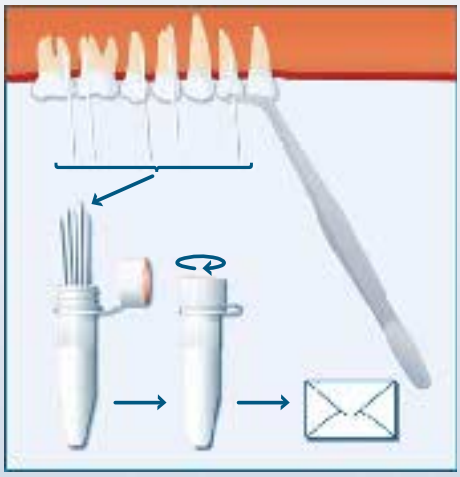


Fig 4

Transport of Samples
The processing and evaluation of the probes is carried out by designated laboratories. Put the samples and the completed order form in the provided envelope and send it to a laboratory performing the **microDent®** test. Since the assay is based on the analysis of nucleic acids no special precautions are required during transport. However, transportation taking several days (e.g . over a weekend) should be avoided, especially during summer. Store the sample in a refrigerator until rapid transport can be ensured.

As a matter of course, results are delivered by post within 3-5 days. Rapid delivery of the results by fax or email is also available. Sets for taking specimens will be provided by the laboratory free of charge.

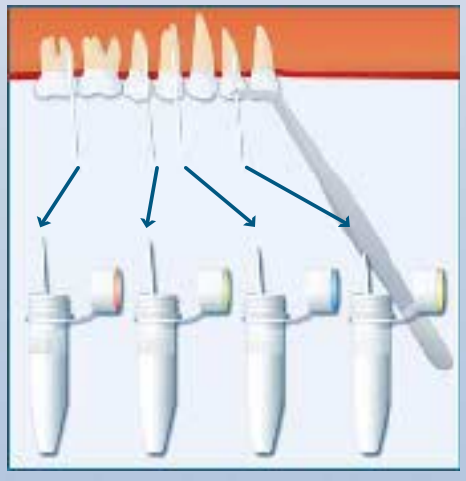


Fig 5

More detailed information and therapy recommendations are available direct from Hain Lifescience.

Periodontitis is Hereditary

Dental practitioners are constantly encountering patients who, despite excellent oral hygiene and a low rate of periodontalpathogenic bacteria, exhibit a strong degradation of the jawbone. Conventional forms of therapy such as cleaning the gingival pockets etc. often, experience has shown, fail to be effective. An American research team (Kornman *et al.* 1997) was able to show for the first time that these patients to a disproportion extent (> 50 %) had a genetic defect in a certain component of the immune system. This leads to over-production of an important local inflammatory mediator in the immune system, namely interleukin-1 (IL-1). The over-production of IL-1 leads to a strong immuno-response in the bone and connective tissue even when only small amounts of bacteria are present. As a result, a hyper-activation of so-called osteoclasts can be detected which themselves then cause an aggressive bone resorption (see Fig 6)
A study by McGuire and Nunn (1999) showed that where the risk genotype is present, the probability of losing teeth or implants during the maintenance period is significantly increased. In combination with heavy smoking, another risk factor, a 10-fold increase in risk was indicated. It is accepted today that the combination of smoking and the presence of the risk genotype definitely leads to an increased risk of implant complications or loss thereof (see Fig 8).

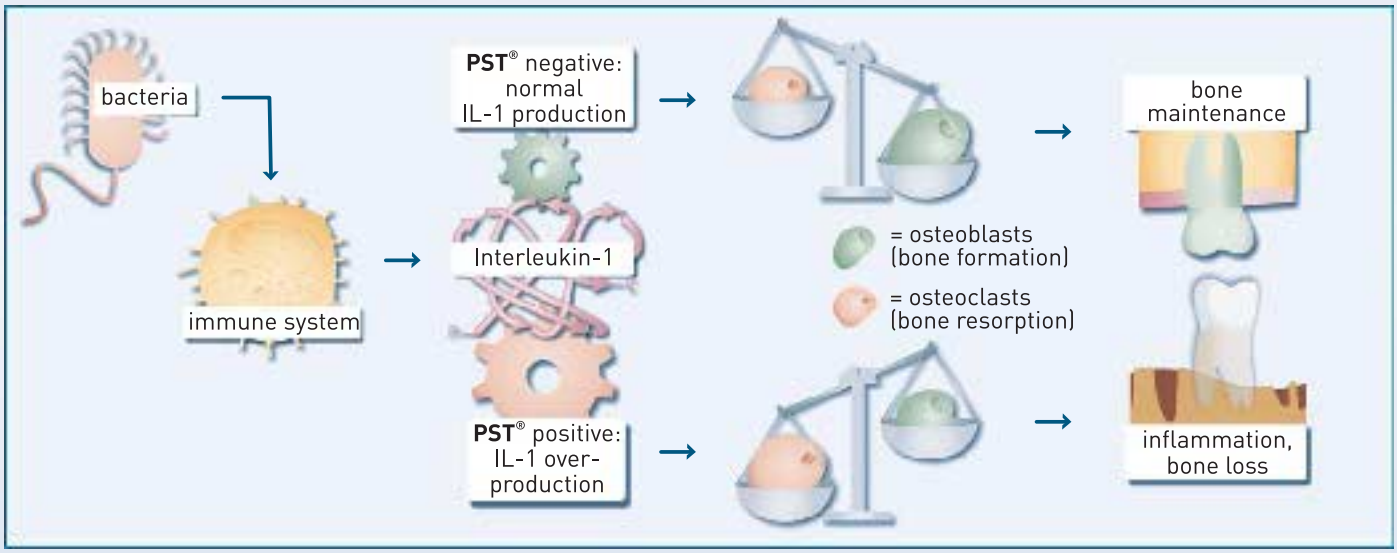


Fig 6

Genetic Principals of the GenoType® PST®:

Two polymorphisms within the IL-1 gene cluster show a close association with periodontitis:
1. Interleukin 1A gene, position -889
2. Interleukin 1B gene, position +3953

Within both polymorphisms allele 1 harbors a cytidin (C), whereas allele 2 carries a thymidin (T) at the respective position. In particular, when both genes carry allele 2 a strong over-production of the local inflammatory mediator, interleukin-1 will occur.
The **GenoType® PST®** detects the corresponding allele combination in patients allowing an evaluation of the individual periodontitis risk and future strategies for therapy.

Indications for the GenoType® PST® test:

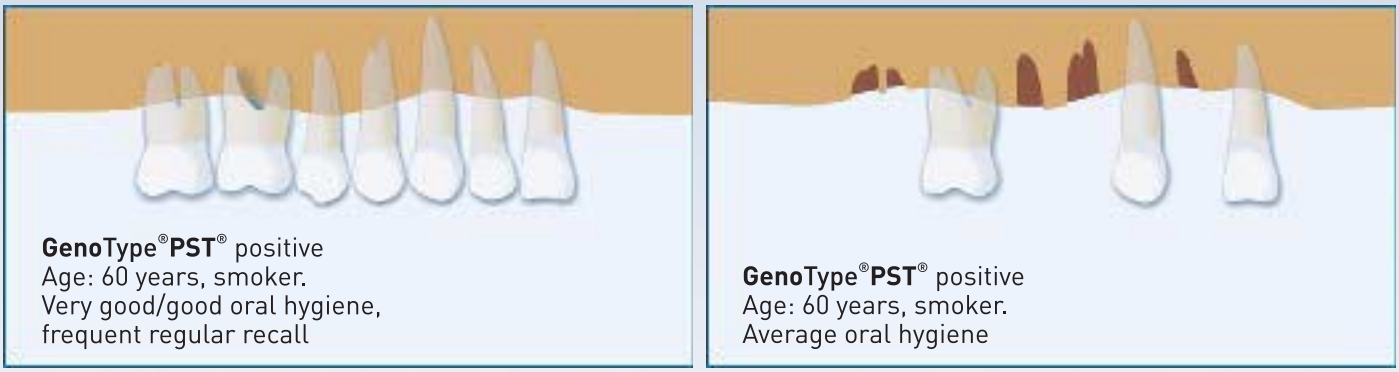
with:
- patients exhibiting agressive, therapy-resistant periodontitis for individual therapy planning
- patients with established periodontitis and loss of attachment for progress assessment
- relatives of **PST®**-positive patients

for:
- risk assessment before major restorative therapy
- optimization of prophylaxis and recall interval

Sound Diagnosis
↓
Reliable Prognosis

Advantages of a Genetic Risk Test

The knowledge of a patients IL-1 genotype, in combination with the individual bacterial load (**microDent®**) together with a consideration of other risk factors like smoking, provides a sound basis for an informed prognosis of the course of the disease and, therefore, also the risk of further teeth or implant loss. On this basis, the dentist or periodontologist is able to draw up an individual therapy plan meeting the needs of the patient (see Fig 7).



GenoType®PST® positive
Age: 60 years, smoker.
Very good/good oral hygiene,
frequent regular recall

GenoType®PST® positive
Age: 60 years, smoker.
Average oral hygiene

Fig 7

Interleukin-1 and implant failure

Various recent studies, carried out in the US and Switzerland, concerning implant failure (Gruica *et al.* 2002; Feloutzis *et al.* 2002) show that smokers, in paticular, who have tested positive for IL-1 mutations have a higher risk of experiencing implant loss. Up to 50% of **PST®**-positive smokers had implant complications, with attachment loss in recall being 3 times higher. It is highly recommended that smokers who have experienced tooth loss as a result of periodontitis should be tested for the IL-1 genotype, not least for the security of the implant practioner. **GenoType® PST®**-positive patients, who are smokers and who do not stop smoking or decline a regular recall (f. ex. four times a year plus prophylaxis), might only be granted a limited guarantee for implant therapy. Smokers who test negative for genetic IL-1 mutations should, at the least, attend an intensive recall, while non-smokers who are genetically negative only require a recall at long intervals (one or two time per year). Thereby, a modern system of quality management can be achieved within the implantology field and economically sound success rates maintained.

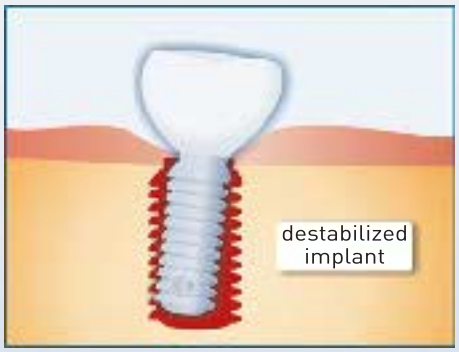


Fig 8

Specimen Collection for the GenoType® PST® Test

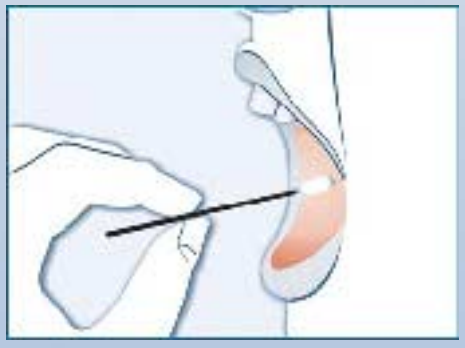


Fig 9

Sample collection is performed by a dentist or a dental hygienist. The following points should be observed.

1. Provide the following information at the appropriate place on the order form: Practice address, patient information, date, clinical diagnosis, other important information such as risk factors etc. together with insurance details.
2. For sampling, take the sterile buccal swab out of the tube and firmly rub it over the mucous membrane of the patient's cheek for 20-30 seconds (see Fig 9).
3. Let the swab dry in air for about 1 minute and place in the appropriate transport tube. Put the sample and the order form in the envelope provided and send it to your laboratory.
4. As a matter of course, results are delivered by post within 3-5 days. Rapid delivery of the results by fax or email is also available.

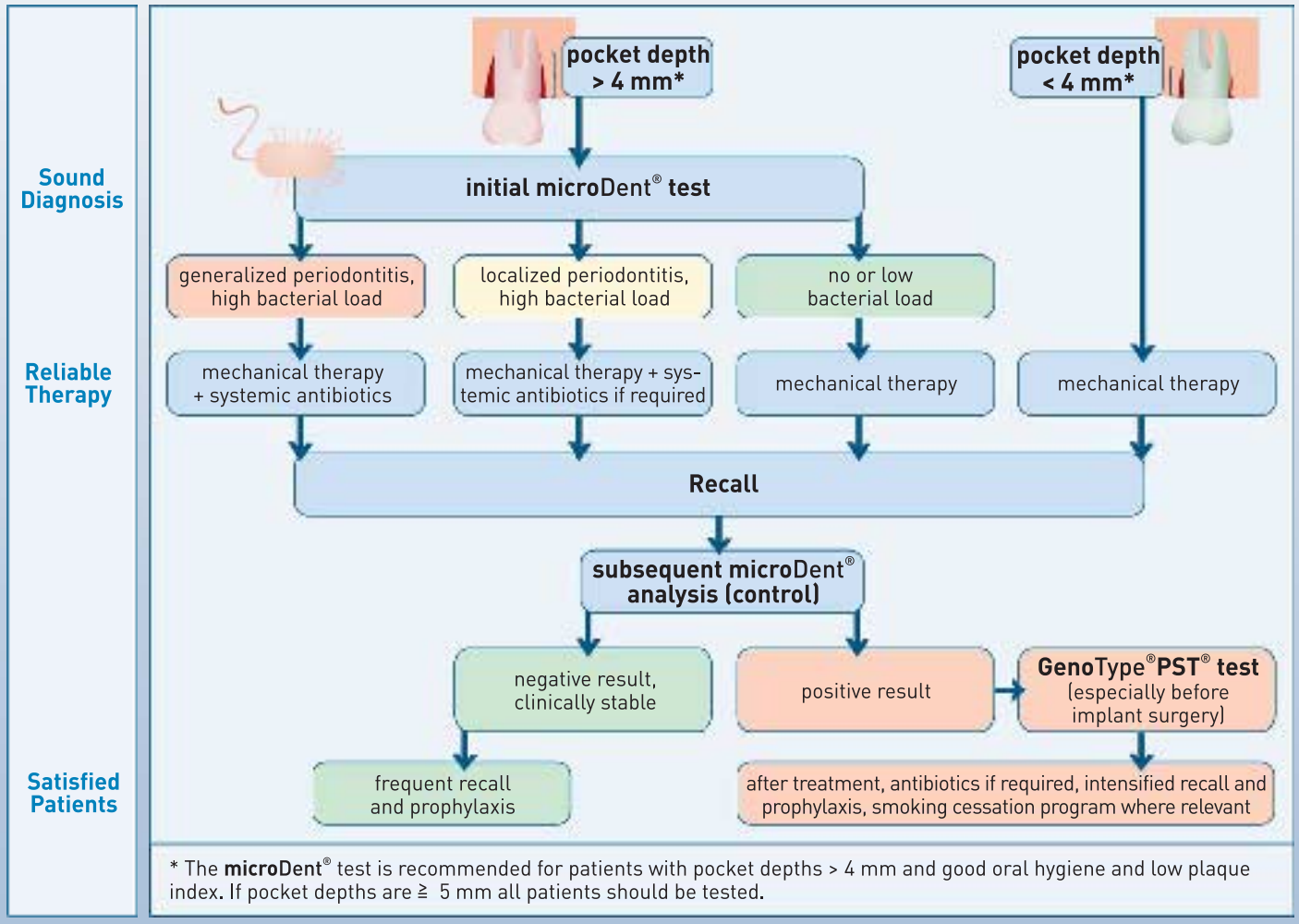
Further details, recommendations for therapy and information for recall strategies can be obtained directly from Hain Lifescience.

Therapy of Periodontal Disease

Where tissue-invasive, periopathogenic bacteria such as *P. gingivalis, A. actinomycetemcomitans* etc. are present, mechanical methods like root-planing or deep-scaling are often ineffective in eliminating the pathogen. Despite careful treatment, the result is progressive attachment loss and bone resorption. In such cases, a one-off antimicrobial concomittal therapy – only undertaken after microbiological diagnostics, of course – is much more effective while causing less side effects. The choices of antiviral agent and the form of application are based on the bacterial spectrum and the clinical phenotype of the periodontitis and therefore can accordingly vary a great deal.
In the main, both local and systemic antibiotic applications are available. In the case of a generalized PA disease, an adjuvant systemic therapy is indicated. If the infection focus is limited to individual sites, a local treatment is a sensible alternative.
In general, antibiotic therapy should only be carried out in conjunction with a careful curettage, where the beginnning of the medication will be scheduled after completed mechanical treatment. Antibiotic therapies should in any case (see DGP recommendations) only be implemented after microbiological diagnostics (e.g. **microDent®**) have been completed, in order to avoid both excessive and under treatment.

Extensive recommendations for therapy can be obtained from Hain Lifescience.

Periodontitis: Concept for Diagnosis and Therapy



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