COMPARISON OF REAL-TIME PCR AND DNA-STRIP TECHNOLOGY IN DETECTION OF PERIODONTOPATHOGENS

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Objective
Periodontopathic bacteria, among them Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Tannerella forsythia, Treponema denticola and Prevotella intermedia, play a major role in pathogenesis of periodontitis. Further other pathogens, e.g. Prevotella intermedia, Parvimonas micra, Eubacterium nodatum, Porphyromonas accepta, and Capnocytophaga sp are also involved. The last two periodontopathic bacteria in subgingival plaque are an important indicator of disease activity and success of periodontal treatment.

In the last years, nucleic-acid based methods replaced cultivation as the “gold standard” in microbiological analysis. Moreover, quantitative test systems have been introduced. The purpose of this study was to compare a commercially available semi-quantitative test (microIdent® including microidentplus) detecting the species mentioned above with quantitative in-house real-time PCR.

Material and Methods
27 patients with severe chronic periodontitis (age: 31-55 years)
365 subgingival plaque samples obtained from different time-points during treatment

Real-time PCR (in-house)
- A. actinomycetemcomitans
- P. gingivalis
- T. denticola
- T. forsythia
- T. intermedia
- P. intermedia
- C. rectus
- F. nucleatum
- P. micra
- E. nodatum
- C. nigripilis
- E. corrodens
- E. corrodens (accession AF543295)
- C. rectus (accession U13404)
- T. forsythia (accession L14636)
- T. forsythia (accession U13404)
- E. corrodens (accession AF543295)

DNA extraction
- microIdent® incl. microidentplus
- PCR
- Reverse hybridization
- Scanning of the strips
- Quantification by means of densitometry

Comparison semi-quantitative
Comparison quantitative

Comparison semi-quantitative
Comparison quantitative

Comparison semi-quantitative
Comparison quantitative

Summary and Discussion
In general, in-house real-time PCR was more accurate than the micro-Ident® test, so negative results obtained by real-time PCR should be confirmed by a second run. The results for all 11 bacterial species correlated significantly between the two methods. Higher R-values were obtained for the major pathogens. The numbers of negative samples by one method and a high load by the other method was always below 2%. Nevertheless, micro-Ident® may be less sensitive for P. gingivalis, T. forsythia, T. intermedia and F. nucleatum. In periodontitis, real-time PCR detected more samples positive for T. forsythia, P. micra, C. rectus, E. nodatum and capnocytophaga sp.; which is to discuss in relation to the toxicity of SybrGreen used in real-time PCR and the selected cut-off and the multiplex PCR in micro-Ident® as well as the regions of rDNA where the primers bind to.

In-house real-time PCR is a cheap useful method for large studies. Semi-quantitative DNA-strip technology can be recommended for diagnosis, and control of treatment in dental practice and also in clinical trials.