Sampling of periodontal pathogens by paper points: evaluation of basic parameters

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Running title: Sampling of bacteria by paper points

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Abstract

Paper points are widely introduced to collect subgingival plaque or other oral samples to analyse the microbiota, especially the presence of periodontal pathogenic bacteria like *Actinobacillus actinomycetemcomitans* or *Porphyromonas gingivalis*. In contrast to the high frequency of usage of paper points in oral sampling, very few data are available about the parameters influencing the sampling process. Therefore, we inoculated paper points in four different *in vitro* experiments (6-9 repeats) with standardised suspensions (2 x 10^9 Colony Forming Units/ml) of *A. actinomycetemcomitans* and *P. gingivalis* testing the influence of the

origin (kind) of paper point ("manufacturer"), size (according to the International Organisation for Standardisation ISO 25-80), probing time (5 to 60 sec), and elution time (5 to 60 sec). Sampled bacteria were detected and (semi-)quantified by using 16S rRNA / DNA directed oligonucleotide probes. The bacterial load was categorised and calculations performed with index values ranging between 0 (< 10^3 bacteria) and 9 (> 10^6 bacteria). We found differences in the efficiency for bacterial sampling between the 5 manufacturers tested, expressed in a mean bacterial index (MBI) between 4.4 and 7.8. Paper points of ISO 45 were found to work most efficiently. According to our results, a probing time of 60 sec seems to be optimal, however, shorter times between 10 and 30 sec do not significantly reduce the sampling efficiency. Furthermore, we found an interval of 20 sec best to elute bacteria from the paper points. The evaluation of basic parameters for subgingival plaque sampling by paper points might help to optimise the microbiological based diagnostic in periodontal diseases.