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Re: Nickles K, Scharf S, Röllke L et al. (2015). Detection of subgingival periodontal pathogens – comparison of two sampling strategies. *Clin Oral Invest*, published online 21 July 2015; DOI 10.1007/s00784-015-1530-4.

Dear Professor Schmalz,

One of the many advantages of Google Scholar is certainly the service of being informed about quotation of one's own articles. After one of my papers of 1993 has recently consistently been quoted in a wrong context, I became curious about the very similar aims described in the respective quoting papers and had a closer look.

The above paper by Nickles et al. [1] which has been accepted for publication in *Clin Oral Investig* is the by now last in a series (I had identified at least 5 papers) of the group which deals with sampling strategies for the detection of subgingival periodontal pathogens. What immediately struck me was apparent self-plagiarism of numerous paragraphs, and I want to outline some of them in more detail.

In the paper by Nickles et al. [1], subgingival samples had been pooled from 4 or 6 deep pockets and analyzed in a commercial laboratory for presence of 11 bacteria employing PCR technology. Both, the Introduction and Discussion sections contain numerous sentences and references which are more or less identical in a 5-yr old paper by Wohlfeil et al. [2] which also addressed the detection rates of periodontal pathogens in subgingival plaque samples from untreated periodontitis using either 4 or 6 pooled samples. Microbiological testing was carried out by 16S rRNA gene probes in another commercial laboratory.

In the following table, conspicuous paragraphs of either paper are compared. Note that some additions in the paper by Nickles et al. [1] are given in blue. As the paper is not paginated, I assign page numbers 1-9.

Nickles et al. (2015)	Wohlfeil et al. (2010)
p. 1	**p. 126**
From the approximately 400 bacterial species colonizing periodontal pockets and a further 300 in the rest of the oral cavity [1, 2], some are frequently associated with periodontal destruction. *Aggregatibacter actinomycetemcomitans* (formerly known as *Actinobacillus actinomycetemcomitans*) [3], *Porphyromonas gingivalis*, *Tannerella forsythia*, and *Treponema denticola* are considered periodontal pathogens. *Prevotella intermedia*, *Parvimonas micra*, *Fusobacterium nucleatum*, *Campylobacter rectus*, *Prevotella nigrescens*, *Eikenella corrodens*, and *Capnocytophaga sp*. are associated with periodontal disease [4, 5].	From the approximately 400 bacterial species colonizing periodontal pockets, and a further 300 in the rest of the oral cavity,[1,2] some are frequently associated with periodontal destruction. *Aggregatibacter actinomycetemcomitans*,[3] *Porphyromonas gingivalis*, *Tannerella forsythia*, and *Treponema denticola*[4,5] are considered periodontal pathogens.
Further, *A. actinomycetemcomitans* has been closely associated with the aetiology of severe periodontal disease: aggressive periodontitis (AgP) [6-10] and periodontitis as manifestation of Papillon Lefevre syndrome [11]. *A. actinomycetemcomitans* is a microaerophilic, facultative anaerobic and Gram-negative coccoid rod belonging to the family of *Pasteurellaceae* [3]. Periodontal disease associated with *A. actinomycetemcomitans* in many cases cannot be treated reliably and predictively by mechanical removal of the subgingival biofilm alone [6, 12-17].	Further, *A. actinomycetemcomitans* has been closely associated with the etiology of a severe periodontal disease, aggressive periodontitis (AgP)[6-10] and periodontitis as manifestation of Papillon-Lefévre syndrome.[11] *Aggregatibacter actinomycetemcomitans* is a micro-aerophilic, facultative anaerobic and Gram-negative coccoid rod belonging to the family of *Pasteurellacea*.[3] Periodontal disease associated with *A. actinomycetemcomitans* in many cases cannot be treated reliably and predictively by the mechanical removal of the subgingival biofilm alone.[6,12-17]
Thus, the detection of *A. actinomycetemcomitans* is a significant factor contributing to the decision whether mechanical anti-infective therapy shoud be used in conjunction with systemic antibiotics [18, 19]. Further, depending on the microbial complexes that are detected from subgingival plaque, it has been proposed to apply varying antibiotic regimens [20].	Thus, the detection of *A. actinomycetemcomitans* is a significant factor contributing to the decision as to whether subgingival debridement should be performed in conjunction with systemic antibiotics.[18,19]
p. 4	**p. 129f**
In the present study, paper points were used for microbiological sampling. This is a standard for commercially available bacteriological tests and was also used in	In the present study, paper points were used for the microbiological sampling. This is standard for commercially-available gene probe tests, and was also used in many

many microbiological studies [9, 15, 22, 33].	microbiological studies.[9,15,20,21,26,34,35] Thus, after investigating a commercially-available 16S rRNA gene probe test, we used paper points for sampling subgingival plaque.
However, paper points have some disadvantages: If the become wet by gingival crevicular fluid, they lose stiffness making it difficult to move them to the bottom of the pocket. Jervoe-Storm et al. compared the recovery of six periodontal pathogens by paper point samples from different aspects of the lesion (full-length or half-length of the pocket depth) in 20 patients: The authors found out that the recovery of the target pathogens was similar following sampling at various depths of the periodontal lesions [34].	However, paper points have some disadvantages: if they become wet by gingival crevicular fluid, they lose stiffness, making it difficult to move them to the bottom of the pocket.
Another disadvantage is that different paper points placed in the same pocket at the same time time and for the same period of time may not sample the same microorganisms [22]. Subgingival plaque may be sampled also using curettes [24]. Curettes are made of steel and consequently stay stiff. Further, a curette samples subgingival plaque from a larger area than a paper point. Thus, sampling plaque with a curette might overcome some of the disadvantages of paper points.	Another disadvantage is that different paper points placed in the same pocket at the same time and for the same period of time might not sample the same microorganism.[26] Subgingival plaque might also be sampled using curettes.[23] Curettes stay stiff, even when they become wet, and can sample subgingival plaque from a larger area than a paper point. Thus, sampling plaque with a curette might overcome some of the disadvantages of paper points.
Jervøe-Storm et al. compared the curette and paper point sampling technique using quantitative real-time PCR [35]. The results demonstrated that the plaque composition with regard to total target pathogens was similar for both sampling techniques.	Jervøe-Storm *et al.* compared the curette and paper point sampling technique using quantitative real-time PCR and demonstrated that the plaque composition, with regard to total target pathogens, was similar for both sampling techniques.[36]
p. 6f	**p. 130**
A. actinomycetemcomitans may not be present at all oral sites in a patient suffering from untreated periodontitis. Taking subgingival samples from all teeth would be the most reliable way to detect A. actinomycetemcomitans. However, this method is too time consuming and expensive to be used in daily practice. Sampling of the deepest pocket of each quadrant has been demonstrated to detect quite reliably the subgingival presence of *A. actinomycetemcomitans* [15] or *P. gingivalis* [33] in untreated patients. Mombelli et al. sampled and microbiologically analyzed all sites separately and theoretically evaluated different sampling strategies [15, 33]. Yet, their strategies were based on separate analyses of the samples, whereas in daily	*Aggregatibacter actinomycetemcomitans* might not be present at all oral sites in a patient suffering from untreated periodontitis.[15,22] Taking subgingival samples from all teeth would be the most reliable way to detect *A. actinomycetemcomitans*. However, this method is too time consuming and expensive to be used in daily practice. Sampling of the deepest pocket of each quadrant has been demonstrated to quite reliably detect the subgingival presence of periodontal pathogens in untreated patients.[15,20,22] Mombelli *et al.* sampled and microbiologically analyzed all sites separately, and theoretically evaluated different sampling strategies.[15,20,21] Yet their strategies were based on separate analyses of the samples, whereas in daily

practice, the different samples taken from the deepest sites per quadrant are pooled prior to analysis for economic reasons. Haffajee et al. analyzed the effect of sampling strategy on the false-negative rate for detection of selected subgingival species. They compared plaque samples obtained from one site per tooth with samples taken from (a) one maxillary molar, (b) both maxillary first molars, (c) four first molars, (d) six Ramfjord teeth, (e) the deepest pocket, and (f) the four deepest pockets. The highest rates of detected species were found for sampling strategy – (f) the four deepest pockets [38]. Thus, for microbiological analysis in daily practice sampling, the deepest site per quadrant generally is recommended [21].	practice, the different samples taken from the deepest sites per quadrant are pooled prior to analysis for economic reasons. Haffajee *et al.*[22] analyzed the effect of sampling strategy on the fals-negative rate for detection of selected subgingival species. They compared plaque samples obtained from one site per tooth with samples taken from one maxillary first molar, the maxillary first molars, the four-first molars, six Ramfjord teeth,39, the deepest pocket, and the four deepest pockets. The lowest rates of undetected species were found for the four deepest pocket sampling strategy.22 Thus for microbiological analyses in daily practice, sampling the deepest site per quadrant is generally recommended.
After sampling, one to six sites per patient and separate analysis per site Beikler et al. reported increasing probability to detect the targeted bacteria with increasing number of sampled sites [24]. However, other authors reported contradicting observations [39]. These differences may be explained by significant differences in methodology: (a) sampling with curettes [24] or paper points [40], and (b) separate [24] or pooled analysis [40]. Thus, at least for pooled analysis up to now, it was not clear whether sampling the deepest sites per sextant (MT6) instead of the deepest per quadrant (MT4) increases the probability of detection. For the PCR, DNA probe test kit that was investigated MT6 failed to demonstrate advantages over MT4. …	After sampling one to six sites per patient, and with a separate analysis per site, Beikler *et al.* reported increasing the probability to detect the targeted bacteria with an increasing number of sampled sites.[23] However, other authors reported contradicting observations.[40] These differences might be explained by significant differences in methodology: sampling with curettes[23] or paper points[40] and separate[23] or pooled analyses[40]. Thus, at least for pooled analyses, up until now it has not been clear whether sampling the deepest site per sextant (MT6) instead of the deepest per quadrant (MT4) increases the probability of detection. For the 16S rRNA gene probe test kit that was investigated in this study, the MT6 sampling strategy demonstrated advantages over MT4, particularly in the detection of *A. actinomycetemcomitans*. …
The studied population in this present study corresponds to the populations analyzed in previous studies [9, 22] regarding patient parameters (age, gender) and clinical parameters (PD, PAL-V). In the present study, *A. actinomycetemcomitans* was detected in 22 % (MT4) and 24 % (MT6) of all patients, with a proportion of patients with aggressive periodontitis: 29 patients (29 %). There is a body of evidence that the prevalence of *A. actinomycetemcomitans* is higher in patients with aggressive periodontitis than in patients with chronic periodontitis [9, 22, 42].	The studied population corresponds to the populations analyzed in previous studies[9,26] regarding patient parameters (age, sex) and clinical parameters (PD, CAL-V).

…	…
P. gingivalis, T. forsythia, T. denticola, P. micra, F. nucleatum, C. rectus, and *E. corrodens* were detected in the majority of the patients (detection frequency of approximately 80 % and higher). *P. intermedi*a was prevalent in 46 %, *E. nodatum* in 65 %, and *Capnocytophaga* sp. In 58 % of the cases. These detection frequencies are in accordance with observations previously made by others using different microbiological tests in patients with untreated aggressive and generalized severe chronic periodontitis study [9, 22, 43]. Thus, in untreated aggressive and generalized severe chronic periodontitis detection of *P. gingivalis, T. forsythia, T. denticola, P. micra, F. nucleatum, C. rectus*, and *E. corrodens* is no essential information because it may be expected in approximately 80-100 % of its kind of patients any way.	*Porphyromonas gingivalis*, *T. forsythia*, and *T. denticola* were detected in almost all patients (*P. gingivalis*: 98-100%, *T. forsythia*: 100%, and *T. denticola*: 98%). These detection frequencies are in accordance with observations previously made by others in patients with untreated AgP and ChP.[9,26,29] Thus, in untreated AgP and ChP, the detection of *P. gingivalis*, *T. forsythia*, and *T. denticola* is not considered to be essential information because it could be expected in more than 95% of patients.

As nearly identical paragraphs comprise 40-50% of the Introduction and Discussion sections, there is apparently more. In their Introduction, Nickles et al. [1] write, "The higher the number of sites [sampled], the higher the detection rate and the less the variability [9, 22, 24]. Sampling six sites instead of four sites increases the effort. Up to now, the comparison of pooled samples from four to six sites has not been investigated."

The respective paragraph in Wohlfeil et al. [2] of 2010 reads (p. 127), "Sampling one to six sites per patient increases the probability of detecting the targeted bacteria.[23] However, sampling six sites instead of four sites increases the cost of diagnosis. Until now, the comparison of pooled samples from four to six sites has only been investigated for polymerase chain reaction (PCR)-based microbiological analyses.[24]" Reference 24 is an abstract by the group which I have not identified yet. As Nickles et al. [1] compare PCR results, their claim ("Up to now, …") must be anyhow regarded false.

Very recently, Ramich et al. [3] of the same research group dealt with the question whether subgingival sampling after treatment should be done at the same sites as before therapy or from residual deep pockets. The Introduction section of the paper by Nickles et al. [1] starts with almost the same sentences, *verbatim*, as those in the Introduction in Ramich et al. [3] and most references are repeated. The same sentences and references are found in the Introduction of an article by Schacher et al. [4] and Krigar et al. [5], also from the same group.

Further self-plagiarism of older articles in Nickles et al. [1] applies, for example, to the long paragraph starting with "*A. actinomycetemcomitans* may not be present at all sites in a patient suffering from untreated periodontitis …" which can be found, *verbatim*, in both Schacher et al. [4] and Krigar et al. [5]; and "Subgingival plaque may be sampled also using curettes …" (*verbatim* in Krigar et al. [5]).

Given that numerous paragraphs in the Introduction and Discussion of the paper by Nickles et al. [1] have been plagiarized from previous, very similar, work (in fact, I am afraid that the series is to be continued infinitively with slightly modified research questions in mind), and grave misconceptions of properties and pitfalls of the kappa statistic (to which I do not want to further comment) when in fact diagnostic test

parameters, in particular false-negative rates would have been appropriate to report, I wonder whether this particular article should be retracted.

A final point regards statements of no conflict of interest in all papers referred to below. In general, companies were acknowledged for providing the tests (for free?). In that case, there is always a conflict of interest and claims otherwise should be disapproved. Moreover, test results had never been validated independently, in particular the conspicuous finding of nearly omnipresence of certain bacteria of the so-called red complex in, for instance, Nickles et al. [1] and Wohlfeil et al. [2].

Interested in receiving your kind response,
Yours sincerely,

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References

1. Nickles K, Scharf S, Röllke L, Mayer I, Mayer M, Eickholz P (2015) Detection of subgingival periodontal pathogens – comparison of two sampling strategies. Clin Oral Investig. DOI 10.1007/s00784-015-1530-4.
2. Wohlfeil M, Tabakci O, Arndt R, Eickholz P, Nickles K (2010) Detection rates of presumptive periodontal pathogens in subgingival plaque samples of untreated periodontitis using either four or six pooled samples. J Invest Clin Dent 1: 126-132.
3. Ramich Tm Schacher B, Scharf S, Röllke L, Arndt R, Eickholz P, Nickles K (2015) Subgingival plaque sampling after combined mechanical and antibiotic nonsurgical periodontal therapy. Clin Oral Investig 19: 27-34.
4. Schacher B, Baron F, Rossberg M, Wohlfeil M, Arndt R, Eickholz P (2007) Aggregatibacter actinomycetemcomitans as indicator for aggressive periodontitis by two analyzing strategies. J Clin Periodontol 34: 566-573.
5. Krigar DM, Kaltschmitt J, Krieger JK, Eickholz P. Two subgingival plaque-sampling strategies used with RNA probes. J Periodontol 78: 72-78.