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ORIGINAL ARTICLE

Clinical evaluation of a newly developed chairside test to determine periodontal pathogens

Nicole B. Arweiler¹ | Vanessa K. Marx¹ | Oliver Laugisch¹ | Anton Sculean² |

Thorsten M. Auschill¹

¹Department of Periodontology & Peri-implant Diseases, Philipps-University, Marburg, Germany

²Department of Periodontology, University of Bern, Bern, Switzerland

Correspondence

Prof. Dr. Nicole B. Arweiler, Department of Periodontology and Peri-implant Diseases, Philipps-University Marburg, Georg-Voigt-Straße 3, 35039 Marburg, Germany Email: arweiler@med.uni-marburg.de

Abstract

Background: The subgingival microbiota as well as determination of markers such as associated pathogens is still in the focus of dental research. The aim of this controlled clinical trial was to determine clinical applicability of a newly developed chairside bacterial test (CST) for the most relevant periodontal pathogens.

Methods: Within 125 participants (100 with periodontitis, 25 healthy) two sulcus fluid samples each were collected and pooled for further analysis. Samples were analyzed with CST and results (positive signals for every pathogen/control) were visually detected by eye. As a reference quantitative polymerase chain reaction (qPCR) was performed.

Results: The detection limit of CST revealed 1.2×10^4 for *Treponema denticola* (*T.d.*) and *Tannerella forsythia* (*T.f.*), 2.5×10^4 for *Porphyromonas gingivalis* (*P.g.*), 5.3×10^3 for Prevotella intermedia (P.i.), and 5.8×10^4 for Aggregatibacter actinomycetemcomitans (A.a.). Based on this maximum potential of positive detections, the sensitivities of CST in reference to qPCR were: T.d. (91.3%); T.f. (86.3%); P.g. (83.8%); P.i. (85.7%), and A.a. (100%). In regard to the clinical diagnosis, the CST assay and the qPCR method reached a sensitivity of 87.82% and 94%, respectively. The specificity for both methods was 100%.

Conclusion: This newly developed CST can detect five typical periodontal pathogens with a somewhat lower sensitivity towards qPCR that can be classified as "good."

KEYWORDS

diagnosis, microbiology, PCR, periodontal disease, periodontitis, point-of-care

1 | INTRODUCTION

Based on current scientific knowledge, various types of periodontal pathogens are of significance in disease initiation and progression, and therefore, for its treatment and prognosis. While a plethora of bacteria (>700 bacterial species) colonize the subgingival area, key species were identified having a high evidence for a striking association with periodontal disease (pocket depth and bleeding on probing [BOP]) such as Aggregatibacter actinomycetemcomitans (A.a.), Porphyromonas gingivalis (P.g.), Tannerella forsythia (T.f.), Treponema denticola (T.d.), and Prevotella intermedia

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(P.i.).^{1,2} These bacteria are also often associated with other species, which results in a complex network of metabolic processes leading to an increase in its pathogenicity.^{2,3}

The usefulness of microbiological testing is the subject of a very controversial debate among scientists. Critics point out that it is not a requirement for proper treatment and patients benefited from antibiotics irrespective of the knowledge of the bacterial status.⁴⁻⁶ Especially the fact of being A. actinomycetemcomitans-positive or -negative did not change the effect of the antibiotics.⁴ Proponents invoke that tests are a useful tool in finding the optimal and individualized treatment option to patients, in particular, when used on patients with (former classified) severe chronic or aggressive periodontitis who do not respond favorably to conventional mechanical therapy.⁷⁻⁹ Studies presenting trends in antibiotic use and presenting some inappropriate prescriptions of second choice antibiotics and emerging antibiotic resistance emphasize that specific microbiological diagnostic tests can be supportive for clinical practitioners.¹⁰ Furthermore, a recent study presented clinical findings that (appear to) support the use of microbiological testing to strengthen the clinical decision for either using or not using systemic antibiotics in conjunction with non-surgical periodontal therapy.¹¹ Considering World Health Organization (WHO) and European Union (EU) statements¹² (summarized in ref. 13) that resistance to common bacteria has reached alarming levels and also dentists have to reconsider their antibiotic use and adopt proactive and rational strategies (such as 1) avoidance of unnecessary use of any antibiotic, 2) use of narrow-spectrum antibiotics whenever possible, and 3) avoidance of broad-spectrum or combination antibiotics besides in cases of severe infections that do not respond otherwise); microbiological diagnosis is advised before selecting antibiotics against infectious diseases in general and periodontal diseases in particular.^{3,12–14}

A recent systematic review⁶ contrasts all the pros and cons and even considers a personalized approach to therapy which includes a targeted microbial approach for the elimination of periodontopathogenic bacteria based on baseline microbial profiles as proposed already decades ago.¹⁵ They even state that the new post-aggressive periodontitis/chronic periodontitis¹⁶ baseline subgingival microbial profiling could become a useful tool for decision on adjunctive antibiotic use.

Finally, they found no support for this approach on their meta-analysis data and conclude that they can not support nor completely disregard baseline detection of periodontopathogenic bacteria.

While it was shown in previous studies that microbiological testing itself could lead to more antibiotic prescription,¹⁷ all authors are in agreement that clinicians should not empirically and not indiscriminately select antibiotic treatment regimens, especially for patients with mild-to-moderate periodontitis.^{4,10,11,18} While antibiotics were initially proposed to treat periodontitis caused by bacteria that have the ability to attach strongly to and penetrate periodontal tissues or dentin tubules and are difficult to be removed mechanically (such as *A.a.* and *P.g.*),^{3,19} antibiotic regimens were later extended to periodontitis with other microbiological profiles since they could also benefit especially in pockets ≥ 6 mm.^{4,20}

For the clinical practitioner not very experienced with the different forms of periodontitis, microbiological testing can be a helpful tool for quality assurance and can help choose a personalized treatment approach. In this context it is important that positive results of microbiological testing do not necessarily imply antibiotic prescription, since most of subgingival bacteria can successfully be reduced by mechanical treatment only.²¹

Many microorganisms largely present in periodontal pockets are not cultivable,^{22,23} and standard microbiological cultivating procedures are unsuccessful. Other immunological and molecular procedures such as quantitative polymerase chain reaction (qPCR) for determination of bacterial DNA have gained more and more importance. These procedures are of higher sensitivity and specificity and require no live bacterial cultures. Collected samples are sent to an external laboratory and require 4 to 7 days for processing. Therefore, a disadvantage of these methods is that appropriate treatment planning and measures (e.g., targeted antibiotic treatment) occur with delay. A faster processing and immediate assessment of the sample directly at the patient (chairside) would save time.

Such so called point-of-care (POC) tests are already established in general medicine for blood coagulation, cardiovascular, and immunological markers, as well as for the analysis of urine. Especially pregnancy, blood glucose and recently also HIV tests are available for domestic use.²⁴ They can help improve the efficiency of health care and reduce their costs.²⁵

In dentistry, POCs still play a tangential role, although many authors underline the importance of, for example, a chairside aMMP-8 test for monitoring disease progression and adapting preventive and therapeutic measures.^{26–29} The aMMP-8 test is inexpensive, easy to use (the results are automated and, therefore, independent of the practitioner's experience), and currently available for routine use by dental and medical professionals linking these disciplines.²⁷ The authors point out, that additional work, especially on the prognostic value of biomarkers in periodontal and peri-implant diseases, is still required.

Recently it was proposed that the combination of biomarkers (such as MMP-8) with bacterial determination (such as *P. gingivalis*) could result in a promising diagnostic or monitoring tool for periodontitis.^{26,28}

A further, newly developed "chairside test" (CST) enables the detection of five periodontal pathogens in a time span of ≈ 20 minutes, but to date no data on CST's sensitivity and specificity is available. Therefore, the aim of this clinical study was to determine the detection level of CST, its sensitivity and specificity for the detection of the five most relevant periodontal pathogens and compare the results with qPCR as reference. Secondary aims were the investigator reliability as well as the relationship to the clinical situation.

2 | MATERIALS AND METHODS

2.1 | Study center and subjects

A sample-size calculation by *ACOMED Statistik** revealed that 100 periodontally diseased and 25 periodontally healthy individuals needed to be included in this study. The necessary sample size was evaluated using simulations of probit analysis for estimation of limit of detection (LoD) when the samples are measured in duplicates. A width of the 95% confidence interval (CI) of about 0.5 units can be established, when a sample size of 50 is used. Taking into account low prevalences of some of the targets, a larger sample size of 100 was chosen. Twenty-five healthy subjects are efficient to estimate an assumed 100% specificity with a 12% width of the lower one-sided Pearson-Clopper 95% CI.

Subjects were selected from the pool of patients at the Department of Periodontology (diseased group) while healthy subjects included staff and students of the Marburg Dental Clinic, Germany.

2.2 | Study population and inclusion criteria

All participants were divided into a periodontally diseased and a healthy group by means of the following inclusion criteria: 1) age \geq 18 years, 2) no intake of antibiotics within the previous 6 months, and 3) no treatment with antibacterial mouthwashes in the last 6 months.

"Periodontally diseased" study participants (with moderate-to-severe periodontitis according to the 1999 International Classification³⁰) had at least two pockets with probing depths \geq 5 mm and positive BOP.

"Periodontally healthy" study participants (control group) had probing depth of 1 to 3 mm and no negative BOP.

Upon written informed consent a total of 125 participants (59 females, 66 males) at an average age of 52.2 \pm 16.9 years were included. One hundred patients (44 females and 56 males; 57.4 \pm 13.7 years) were classified as "periodon-tally diseased." The control group consisted of 25 "periodon-tally healthy" participants (15 females, 10 males) at an average age of 31.3 \pm 11.23 years.

2.3 | Study design and procedure

This single-center, prospective, case-controlled study (Fig. 1) followed the current guidelines of the United States' Food and

Drug Administration (FDA 2007)³¹ and the Clinical and Laboratory Standards Institute (CLSI 2008).³² The investigation of the CST was registered with the corresponding authorities (DIMDI: DE/CA99/5901) and ethic commission as a medical product law (MPG/in vitro diagnostic study). The Medical Ethics Committee at Philips-University Marburg, Germany approved this study (#84/10). All anonymized study participants received a three-digit identification number according to the order of recruitment and independent of "healthy" or "diseased" criteria.

2.4 | Sample collection and implementation of the chairside test

Sample collection as well as reading the CST chip was performed by the investigators (NA and TA) independently of one another to assess examiners reliability. For each patient, two sampling areas were specified and supragingivally debrided. Using tweezers, a sterile paper point (ISO 35)[†] was then placed into the base of the pocket for ≈ 20 seconds to absorb sulcular fluid (subgingival plaque sample). In healthy participants paper points were inserted 1 to 2 mm into the sulcus, as no periodontal pockets were present. Afterwards, paper points of each patient were pooled and placed into one reaction tube, pre-filled with glass beads. For anonymization, each tube got a sticker with the study participants' identification number and the date the samples were taken. The identification number drew no association to the origin of the sample.

Paper points were immersed in 160 µL of lysis solution, which was added into the reaction tube. The tube was immediately shaken for 30 seconds to extract the bacterial nucleic acids. Samples were then heat-treated for 6 minutes in boiling water. Immediately following this step, 20 µL of this hot sample solution were pipetted into the inlet of each of the test chips and drawn into the sample channel by capillary forces. After 2 minutes of incubation, the following solutions were additionally added into the inlet in the listed order while waiting 2 minutes before adding the next solution: 1) Enzyme solution in preparation of the color reaction; 2) Washing solution to remove unspecifically bound molecules; and 3) Color reaction solution for visualization of hybridization through an enzyme reaction. After waiting an additional 4 minutes, results on test chips were interpreted. A weak or strong blue coloration represented a positive reaction (i.e., bacteria present) (Fig. 2).

The legend of the abbreviated bacteria names (e.g., *T. denticola* = *T.d.*) were imprinted next to the channel on the chip and provided easy identification of the corresponding signal. Additionally, three controls on the chip became visible after a successful test procedure: the C1 signal confirms a successful hybridization (i.e., binding to the probe), the C2 signal the functioning of the enzyme, and the signal related to the total

^{*} ACOMED Statistik, Leipzig, Germany.

[†] DiaDent Group International, Burnaby, British Columbia.



FIGURE 1 Summary of the study design



FIGURE 2 Picture of a test slide of CST

germ load confirms correct sampling and efficient bacterial lysis.

Each sample was tested twice using two CST test chips by two examiners (NA, TA) independently of one another.

2.5 | Quantitative real-time PCR as a method for comparison

The leftover sample lysate ($\approx 120 \ \mu\text{L}$ each) was sealed tightly with Parafilm M,* immediately frozen, and stored at -20° C. Samples were shipped on dry ice to the laboratory[†] and analyzed using quantitative real-time PCR (qPCR) as reference. DNA was extracted using the GenElute Bacterial Genomic DNA Kit[‡] according to manufacturer's protocol and a specific PCR was performed using SYBR Green Supermix.[§] The PCR reaction mixture was prepared in a total volume of 25 μ L, using 12.5 μ L ready-to-use reaction cocktail, 10.5 μ L PCRgrade H₂O, 0.5 μ L forward primer (2 μ M), 0.5- μ L reverse primer (2 μ M), and 1- μ L template. The reactions were set up

^{*} Bemis, Neenah, WI.

[†]Lambda, Rainbach, Austria.

[‡]Sigma-Aldrich, St. Louis, MO.

[§] Sigma-Aldrich, St. Louis, MO.

in 96-well optical plates* and amplified using a C1000 Thermal Cycler with a CFX96 Real-Time System.[†] Cycling conditions were 10 minutes at 94°C; 40 cycles of 30 seconds at 94°C, 40 seconds at 60°C or 63°C or 67°C for *T.d.*, *T. f.*, *P.g.*, *P.i.*, and A.a., respectively, 40 seconds at 72°C, and a final extension at 72°C for 3 minutes. Proprietary primers targeting the 16S rRNA-region to perform species-specific amplification were used. Standardized positive and negative controls were included in the runs. All samples were tested in double.

2.6 | Artificial enrichment of Aggregatibacter actinomycetemcomitans

The prevalence of the bacterium *A. actinomycetemcomitans* (even in periodontally diseased patients) is very low. However, since its incidence is important for the comparison of the detection ability, 16 of 100 samples of the diseased group were artificially enriched with *A. actinomycetemcomitans*. This occurred through the addition of spiked lysis solution, which was masked for the examiner. Eight different concentrations were used to cover a range of bacterial loads. For 16 of the randomly selected patient samples 160 μ L of each "enriched" lysis solution was added (rather than the original). The CST was completed twice. Therefore, every dilution was used two times. This ensured a protocol for the enriched bacterial marker not deviating from that of the clinical test.

2.7 | Data management/data collection forms

Data regarding patient's clinical diagnosis (healthy/diseased) were documented anonymously on data collection sheets. CST results of both examiners were recorded independently. These data as well as the results of the comparison method were transferred into three Microsoft Excel[‡] tables and sent anonymously to ACOMED Statistik[§] who completed statistical data analysis.

2.8 | Statistical analysis

The primary aim of the study was the determination of the detection limit of CST for the five periodontal pathogens each as well as sensitivity and specificity for the detection compared with qPCR as reference. Secondary aims were examiner's reliability and relationship to the clinical situation (periodontally healthy or diseased).

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TABLE 1 Cross tabulation for overall sensitivity and specificity of CST for each investigator and investigators' reliability (Investigator 1, Investigator 2)

	Bacterial detection by CST			
Clinical finding	Positive	Negative	Total (valid)	
Investigator 1				
Diseased	169	29	198	
Healthy	0	50	50	
Sensitivity	169/198	85.35%		
Specificity	50/50	100%		
Investigator 2				
Diseased	169	30	199	
Healthy	0	50	50	
Sensitivity	169/199	84.92%		
Specificity	50/50	100%		
Investigator reliability	,		99.2%	

3 | RESULTS

3.1 | Investigator reliability

Investigator's reliability was tested using 500 results for the determination of the two test chips for each of the 125 participants (investigated independently by two examiners). Investigator 1 could not read two test chips, Investigator 2 could not read one test chip; thus for investigator 1, 198 results from "diseased group" (and 50 "healthy results"), and for Investigator 2, 199 in diseased group (and 50 "healthy results") could be used for evaluation. Both examiners showed identical evaluation in 248 of 250 test chips, the investigator reliability was 99.2% (Table 1).

3.2 | Sensitivity and specificity in relation to the clinical situation

Both CST and qPCR showed a specificity of 100%, which means that no test system found the five pathogens in healthy subjects (Table 2). The sensitivity of CST was 85.14% compared with 94% for reference method qPCR, meaning that both methods did not detect bacteria in all samples of diseased patients.

3.3 | Limit of detection, sensitivity with respect to qPCR, "corrected" sensitivity and bacterial prevalence

The LoD was defined as the concentration from the quantitative determination (qPCR) at which 50% of the CST results are positive. The determination was carried out by probit analysis. LoD was specified together with the corresponding 95% CI. The calculated LoD of the CST for individual bacteria and the percentage of positive detection by qPCR are presented in

^{*} Quanta Bioscience, Gaithersburg, MD.

[†] Bio-Rad, Hemel Hepstead, UK.

[‡] Microsoft Excel, Redmond, WA.

[§] ACOMED Statistik, Leipzig, Germany.

	CST		qPCR		
Bacterial					
detection	Diseased	Healthy	Diseased	Healthy	
Positive	338	0	94	0	
Negative	59	100	6	25	
Sensitivity	85.14% (338/397)		94% (94/100)		
Specificity		100% /100/100)		100% (25/25)	

Cross tabulation for bacterial detection in relation to the clinical findings (groups) (CST: n = 397; qPCR: n = 100) TABLE 2

TABLE 3 Prevalence of the bacteria in diseased group with CST and qPCR, sensitivity of CST, and calculated limit of detection

Bacterial species	Pos. detection CST in %	Pos. detection qPCR in %	Sensitivity CST/ qPCR in % ^a	Calculated detection limit of CST
T. denticola	73	85	85.89	1.2×10^4
T. forsythia	69	94	73.41	1.2×10^4
P. gingivalis	57	74	77.03	2.5×10^4
P. intermedia	24	52	46.16	5.3×10^4
A. actinomycete mcomitans	15 ^b	31 ^b	48.39	5.8×10^4

^aSensitivity in relation to the maximum positive detection of the CST based on the calculated detection limit.

^bArtificially enriched.

Bacterial species	Maximum potential for CST in % ^a	Corrected sensitivity ^a
T. denticola	80	91.3
T. forsythia	80	86.3
P. gingivalis	68	83.8
P. intermedia	28	85.7
A. actinomycetemcomitans	15	100

TABLE 4	Corrected	sensitivity	based	on	maximum	possible
detection of CST	ſ					

^aBased on the calculated detection limit of CST.

Table 3. Thus, the following prevalence was found for CST: T. denticola in 73%, T. forsythia in 69%, P. gingivalis in 57%, P. intermedia in 24%, and A. actinomycetemcomitans in 15% of the samples. Using qPCR, T. denticola was found in 85%, T. forsythia in 94%, P. gingivalis in 74%, P. intermedia in 52%, and A. actinomycetemcomitans in 31% of the samples.

Based on LoD, the maximum number of possible detection of the CST was assessed and resulted in the maximum potential for CST, and thus, in a "corrected" sensitivity compared with qPCR of 91.3% for T. denticola, 86.3% for T. forsythia, 83.3% for P. gingivalis, 85.7% for P. intermedia, and 100% for A. actinomycetemcomitans (Table 4).

4 | DISCUSSION

The aim of the present study was to determine the detection level of a new and innovative CST, its sensitivity and specificity for the detection of the five most relevant periodontal pathogens and compare the results with qPCR as a benchmark reference. Furthermore, precision and accuracy of the test between different users as well as on predicting clinical findings (i.e., periodontally diseased or healthy) were examined. Comparison with qPCR (instead of end-point PCR) was necessary to actually quantify the bacterial load and determine the LoD. The results of other methods such as checkerboard DNA-DNA hybridization or DNA strip technology are at best semi-quantitative and could, therefore, not be used for quantitation.

Mainly A. actinomycetemcomitans, P. gingivalis, T. forsythia, T. denticola, and P. intermedia significantly influence disease initiation and progression, as they are associated in complexes of more or less pathogenicity.^{2,33} Bacterial determination by microbiological testing is important to monitor the disease and was proposed as part of routine diagnostics in severe cases.⁷

In the present study, prevalence of 73%/85% (CST/qPCR) for T. denticola, 69%/94% for T. forsythia, 57%/74% for P. gingivalis, 24%/52% for P. intermedia, and 15%/31% for A. actinomycetemcomitans, respectively, was shown (Table 3). These are in line with other studies using similar inclusion criteria. While Jervoe-Storm et al.²² found 44% for P. gingivalis, 44% (P. intermedia), and 8% (A. actinomycetem*comitans*) with real-time PCR (at a detection level of 10^2), Cosgarea et al.³⁴ identified with PADO* test (detection threshold of 10^3 for A. actinomycetemcomitans and 10^4 for all other bacteria)/MERI^{\dagger} test (detection threshold of 10²) a prevalence of 91%/93% for T. denticola, 91%/96% for T. forsythia, 73%/78% for P. gingivalis, and 19%/25% for A. actinomycetemcomitans. Similar results, using different qPCR methods are provided by Polonyi et al.³⁵ They detected T. denticola in 76%, T. forsythia in 98%, P. gingivalis in 80%, P. intermedia in 33%, and A. actinomycetemcomitans in 10% of

^{*} IAI Pado Test 4.5[®], Zuchwil, Switzerland

[†] Meridol[®] Paro Diagnostik, Gaba GmbH, Lörrach, Germany

all investigated samples. It should be kept in mind that in this present study the amount of *A. actinomycetemcomitans* was artificially enriched by immersing the paper points in defined dilutions. This was necessary to receive enough data to assess and evaluate the potential of CST considering its expected low prevalence.

A specificity of 100% for both CST and qPCR showed that both test systems did not detect the five pathogens in the periodontally healthy study population of the present study, which differs to the findings of Jervoe-Storm et al.²² in a similar study presenting a specificity of only 84% for the qPCR, meaning that in some cases also healthy subjects carried the pathogens.

A sensitivity between 46% (for *P. intermedia*) and 86% (for T. denticola) of the CST compared with the reference method demonstrates that the presence of bacteria could not be verified in all samples that were detected by qPCR. This opens the discussion for the difficulty in finding a proper reference method. Since the qPCR method provides a quantitative result while the CST provides a dichotomous decision (i.e., bacteria present or bacteria not present) a comparison between both methods is of limited extent. The qPCR is accepted as the gold standard and is able to detect extremely small numbers of bacteria (about 500 to 900 bacteria) since it amplifies the results. As there is no other reference method, it has to be considered that qPCR detects also dead bacteria's DNA. The method of the CST is based on RNA detection. It principally detects living bacteria that can proliferate and could cause damage in the periodontal pocket although RNA is more unstable than DNA.³⁰ In this context, Polonyi et al.³⁵ point out that DNA is slowly degraded after vitality loss. Under certain circumstances it might even be detectable years after cell death. Thus, they recommend the use of RNA-based detection methods especially to verify successful eradication of periodontal pathogens.

Hence, it is natural that qPCR is more sensitive, which is reflected in the detection limit of 10^2 in the present study, which is comparable with other studies that used qPCR to detect periodontal pathogens.^{22,34,36} The detection limit of the CST was 10^4 , and therefore, higher than the qPCR. A different rRNA-based test (iai Pado)* detected *A. actinomycetemcomitans* out of 10^3 bacteria and other periodontal pathogens out of 10^4 .^{34,36} In conclusion, the detection limits of the CST are in range to the ones in the PADO test. However, when regarding detection limits it should be kept in mind that also healthy individuals can be colonized by periodontal pathogens and the amount of periodontal pathogens plays a role in developing periodontal disease.

To overcome difficulties when comparing different test systems, a corrected sensitivity was calculated by setting the results in relationship to the maximum potential of the CST. The corrected sensitivity (i.e., the sensitivity within the detection limit of ≈ 1.2 to 5.8×10^4) can be classified as "excellent" with values between 84% and 100% according to a similar study for *A. actinomycetemcomitans* detection where sensitivity of 67% and the specificity of 100% were classified as "excellent."²²

Having high investigator reliability is important for a new test to guarantee that the application is easy to handle. A consensus of 99.2% can be classified as "excellent," a minimal difference could also be explained by a little time-span between read-outs in band intensities.

The newly developed CST is able to detect five typical periodontal pathogens; however, with a higher detection limit than qPCR. For the clinical practice routine, it is discussable if the periodontal therapy needs exact numbers of the bacteria or if the presence or absence of pathogenetic bacteria or better said their virulent RNA is sufficient. A somewhat lower sensitivity than the reference method can still be classified as "good," especially due to the fact that RNA-based methods justifiably detect living bacteria as opposed to DNA-based reference methods.

5 | CONCLUSIONS

CST offers the opportunity to get a quick overview over virulent periodontal pathogens in patients' pockets at the POC with immediate results and could, therefore, provide an advantage over traditional microbiological assessment procedures (e.g., qPCR). CST can at least be a helpful tool in periodontal diagnostics to support the decision to supplement periodontal therapy with or without antibiotics and therewith help to choose the most appropriate and personalized approach to treatment.

Drawbacks of the method are 1) that the results are qualitative (yes or no) and provide only the information that bacteria are present above the calculated LoD and 2) that the visual interpretation of the results might be difficult in some cases although two independent investigators had a high agreement (98.7%). Therefore, a method to objectively identify positive signals, for example, using a software application with an electronic reporting system would be helpful which also could document and store the results.

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^{*} iai Pado, Zuchwil, Schweiz.

REFERENCES

- 1. Colombo AP, Boches SK, Cotton SL, et al. Comparisons of subgingival microbial profiles of refractory periodontitis, severe periodontitis, and periodontal health using the human oral microbe identification microarray. *J Periodontol*. 2009;80:1421-1432.
- Socransky SS, Haffajee AD, Cugini MA, Smith C. Microbial complexes in subgingival plaque. J Clin Periodontol. 1998;25:134-144.
- Mombelli A, Samaranayake LP. Topical and systemic antibiotics in the management of periodontal diseases. *Int Dent J.* 2004;54:3-14.
- Mombelli A, Cionca N, Almaghlouth A, Decaillet F, Courvoisier DS, Giannopoulou C. Are there specific benefits of amoxicillin plus metronidazole in *Aggregatibacter actinomycetemcomitans*associated periodontitis? Double-masked, randomized clinical trial of efficacy and safety. *J Periodontol*. 2013;84:715-724.
- Mombelli A, Almaghlouth A, Cionca N, Cancela J, Courvoisier DS, Giannopoulou C. Microbiologic response to periodontal therapy and multivariable prediction of clinical outcome. *J Periodontol*. 2017;88:1253-1262.
- Nibali L, Koidou VP, Hamborg T, Donos N. Empirical or microbiologically-guided systemic antimicrobials as adjuncts to non-surgical periodontal therapy? A systematic review. *J Clin Periodontol.* 2019. https://doi.org/10.1056/NEJMoa040583
- Loomer PM. Microbiological diagnostic testing in the treatment of periodontal diseases. *Periodontol 2000*. 2004;34:49-56.
- Slots J. Comparison of five growth media and two anaerobic techniques for isolating bacteria from dental plaque. *Scand J Dent Res.* 1975;83:274-278.
- 9. Slots J, Research S, Therapy C. Systemic antibiotics in periodontics. *J Periodontol.* 2004;75:1553-1565.
- Falkenstein S, Stein JM, Henne K, Conrads G. Trends in antibiotic use and microbial diagnostics in periodontal treatment: comparing surveys of German dentists in a ten-year period. *Clin Oral Investig.* 2016;20:2203-2210.
- Eick S, Nydegger J, Burgin W, Salvi GE, Sculean A, Ramseier C. Microbiological analysis and the outcomes of periodontal treatment with or without adjunctive systemic antibiotics-a retrospective study. *Clin Oral Investig.* 2018;22:3031-3041.
- 12. Preus HR, Scheie AA, Baelum V. Letter to the editor: re: the clinical effect of scaling and root planing and the concomitant administration of systemic amoxicillin and metronidazole: a systematic review; Re: effectiveness of systemic amoxicillin/metronidazole as adjunctive therapy to scaling and root planing in the treatment of chronic periodontitis: a systematic review and meta-analysis; Re: effectiveness of systemic amoxicillin/metronidazole as an adjunctive therapy to full-mouth scaling and root planing in the treatment of aggressive periodontitis: a systematic review and meta-analysis. *J Periodontol.* 2014;85:374-384.
- van Winkelhoff AJ, Rams TE, Slots J. Systemic antibiotic therapy in periodontics. *Periodontol 2000*. 1996;10:45-78.
- 14. Veloo AC, Seme K, Raangs E, et al. Antibiotic susceptibility profiles of oral pathogens. *Int J Antimicrob Agents*. 2012;40:450-454.
- van Winkelhoff AJ, Rodenburg JP, Goene RJ, Abbas F, Winkel EG, de Graaff J. Metronidazole plus amoxycillin in the treatment of *Actinobacillus actinomycetemcomitans* associated periodontitis. *J Clin Periodontol.* 1989;16:128-131.
- Tonetti MS, Greenwell H, Kornman KS. Staging and grading of periodontitis: framework and proposal of a new classification and case definition. *J Clin Periodontol*. 2018;45(suppl 20):S149-S161.

- Levy D, Csima A, Birek P, Ellen RP, McCulloch CA. Impact of microbiological consultation on clinical decision making: a casecontrol study of clinical management of recurrent periodontitis. J *Periodontol.* 1993;64:1029-1039.
- Rams TE, Degener JE, van Winkelhoff AJ. Antibiotic resistance in human chronic periodontitis microbiota. *J Periodontol*. 2014;85:160-169.
- Guerrero A, Griffiths GS, Nibali L, et al. Adjunctive benefits of systemic amoxicillin and metronidazole in non-surgical treatment of generalized aggressive periodontitis: a randomized placebocontrolled clinical trial. J Clin Periodontol. 2005;32:1096-1107.
- 20. Guerrero A, Nibali L, Lambertenghi R, et al. Impact of baseline microbiological status on clinical outcomes in generalized aggressive periodontitis patients treated with or without adjunctive amoxicillin and metronidazole: an exploratory analysis from a randomized controlled clinical trial. *J Clin Periodontol*. 2014;41:1080-1089.
- Graziani F, Karapetsa D, Alonso B, Herrera D. Nonsurgical and surgical treatment of periodontitis: how many options for one disease. *Periodontol 2000*. 2017;75:152-188.
- Jervoe-Storm PM, Koltzscher M, Falk W, Dorfler A, Jepsen S. Comparison of culture and real-time PCR for detection and quantification of five putative periodontopathogenic bacteria in subgingival plaque samples. *J Clin Periodontol*. 2005;32:778-783.
- 23. Paster BJ, Boches SK, Galvin JL, et al. Bacterial diversity in human subgingival plaque. *J Bacteriol*. 2001;183:3770-3783.
- Dincer C, Bruch R, Kling A, Dittrich PS, Urban GA. Multiplexed point-of-care testing - xPOCT. *Trends Biotechnol*. 2017;35:728-742.
- Howick J, Cals JW, Jones C, et al. Current and future use of point-of-care tests in primary care: an international survey in Australia, Belgium, The Netherlands, the UK and the USA. *BMJ Open*. 2014;4:e005611.
- Sorsa T, Gieselmann D, Arweiler NB, Hernandez M. A quantitative point-of-care test for periodontal and dental peri-implant diseases. *Nat Rev Dis Primers*. 2017;3:17069.
- Alassiri S, Parnanen P, Rathnayake N, et al. The ability of quantitative, specific, and sensitive point-of-care/chair-side oral fluid immunotests for aMMP-8 to detect periodontal and peri-implant diseases. *Dis Markers*. 2018;2018:1306396.
- Salminen A, Gursoy UK, Paju S, et al. Salivary biomarkers of bacterial burden, inflammatory response, and tissue destruction in periodontitis. *J Clin Periodontol*. 2014;41:442-450.
- Sorsa T, Gursoy UK, Nwhator S, et al. Analysis of matrix metalloproteinases, especially MMP-8, in gingival creviclular fluid, mouthrinse and saliva for monitoring periodontal diseases. *Periodontol 2000*. 2016;70:142-163.
- Armitage GC. Development of a classification system for periodontal diseases and conditions. *Ann Periodontol.* 1999;4:1-6.
- Meier K. Guidance for industry and FDA staff Statistical guidance on reporting results from studies evaluating diagnostic tests. 2007.
- Garrett PE, Lasky FD, Meier KL, Clark LW. User Protocol for Evaluating of Qualitative Test Performance: Approved Guideline. 2nd ed. Wayne, PA: Clinical and Laboratory Standars Institute; 2008.
- 33. Bidault P, Chandad F, Grenier D. Systemic antibiotic therapy in the treatment of periodontitis. *J Can Dent Assoc.* 2007;73:515-520.
- Cosgarea R, Baumer A, Pretzl B, Zehaczek S, Kim TS. Comparison of two different microbiological test kits for detection of periodontal pathogens. *Acta Odontol Scand*. 2010;68:115-121.

 Untch M, Schlagenhauf U. Inter- and intra-test agreement of three commercially available molecular diagnostic tests for the identification of periodontal pathogens. *Clin Oral Investig.* 2015;19:2045-2052. How to cite this article: Arweiler NB, Marx VK, Laugisch O, Sculean A, Auschill TM. Clinical evaluation of a newly developed chairside test to determine periodontal pathogens. *J Periodontol*. 2019;1-9. https://doi.org/10.1002/JPER.19-0180