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The additional value of real-time PCR in the quantitative detection of periodontal pathogens

Khalil Boutaga[1], Arie Jan van Winkelhoff[1], Christina M. J. E. Vandenbroucke-Grauls[2] and Paul H. M. Savelkoul[2]

[1]Department of Oral Microbiology, Academic Center for Dentistry Amsterdam (ACTA), Universiteit van Amsterdam, and Vrije Universiteit; [2]Department of Medical Microbiology and Infection Control, VU University Medical Center Amsterdam, The Netherlands

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Abstract

Background and Aim: For the analysis of subgingival plaque, anaerobic bacterial culture has been the gold standard for many years. Currently, molecular microbial techniques have become available to identify and quantify target organisms with high specificity and sensitivity. The technique of real-time (RT-PCR) provides a new tool to detect oral pathogens both in oral and non-oral human infections. The aim of this study was to compare the RT-PCR and anaerobic culture for detection and quantification of six periodontal pathogens in periodontal health and disease.

Material and Methods: Subgingival plaque samples from 259 adult patients with periodontitis and 111 healthy controls were analysed with quantitative anaerobic culture and quantitative RT-PCR for *Actinobacillus actinomycetemcomitans*, *Porphyromonas gingivalis*, *Prevotella intermedia*, *Tannerella forsythia*, *Micromonas micros* and *Fusobacterium* spp.

Results: All species were more frequently isolated from patients than controls with both culture and RT-PCR. *P. gingivalis, T. forsythia* and *M. micros* appeared significant markers for disease with both techniques. *P. intermedia* was significantly associated with periodontitis by RT-PCR only (OR 9.7), whereas *A. actinomycetemcomitans* showed a significant relationship by culture only. The critical differences between culture and RT-PCR were culture-negative/PCR-positive samples which amounted to 7% for *A. actinomycetemcomitans*, 3% for *P. gingivalis*, 7% for *T. forsythia,* 20% for *P. intermedia*, 6% for *M. micros*, and 0.8% for *Fusobacterium* spp. in periodontitis patients and 12%, 3%, 2%, 35%, 14% and 0%, respectively, in the periodontally healthy group. Furthermore, periodontitis individuals had significantly higher amount of all of the test species in the subgingival plaque samples compared with healthy subjects.

Conclusion: RT-PCR provides a new rapid diagnostic tool and opens the opportunity to detect small numbers of oral pathogens in clinical specimens, which are under the detection limit by culture technique.

Key words: anaerobic culture technique; real-time PCR; periodontitis

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The prevalence of periodontitis in humans is approximately 30%, 10–15% of whom have a severe form of the disease (Brown et al. 1989, Papapanou 1996, Oliver et al. 1998). Risk factors for destructive periodontal disease include presence of bacterial pathogens, smoking, diabetes mellitus and possibly certain genetics traits (Haber et al. 1993, Michalowicz 1994). Periodontitis has been linked to non-oral disorders such as coronary heart diseases (DeStefano et al. 1993), preterm birth (Offenbacher et al. 1996, 1998), juvenile rheumatoid arthritis (Lagervall et al. 2003, Mercado et al. 2003), and dental focal infections (van Winkelhoff et al. 1999). The number of bacterial species that can inhabit periodontal lesions has been estimated >500 (Paster et al. 2001) of which approximately 50% can be cultured. The number of cultivable species that has been associated with disease progression is limited to <10 species and includes *Actinobacillus actinomycetemcomitans*, *Porphyromonas gingivalis*, *Tannerella forsythia*, *Prevotella intermedia* and *Fusobacterium* spp. (Haffajee & Socransky 1994, Griffen et al.

1998). Conventional anaerobic culture technique has been used to detect and quantify marker bacteria for disease progression and to select patients that may benefit from systemic antimicrobial therapy and to evaluate treatment outcome. DNA-based techniques for detection of human microbial pathogens has been introduced in periodontal microbiology and have shown good sensitivity and specificity for some periodontal pathogens (Loesche 1992, Lyons et al. 2000, Boutaga et al. 2003). Rapid and cost-effective microbial diagnosis in cases of severe periodontitis is especially relevant as the introduction of a one-visit treatment protocol has been introduced (De Soete et al. 2001). Comparison of quantitative analysis of the subgingival plaque from a significant number of periodontally healthy and diseased subjects by conventional culture technique and real-timehas not been evaluated yet. Based on the higher sensitivity and specificity of the PCR technique in general (Lau et al. 2004, Nonnenmacher et al. 2004, Boutaga et al. 2005, Jervoe-Storm et al. 2005), one may anticipate a change in view on the occurrence and role of conventional marker bacteria in destructive periodontal disease. The aim of the present trial was to study the detection frequency and the amount of periodontitis-associated marker bacteria by RT-PCR and to evaluate the outcome of this technique with conventional anaerobic culture analysis in periodontal health and disease.

Material and Methods

Patients and clinical samples

The study population included 259 adult patients with periodontitis from whom subgingival plaque samples were collected and sent to the Department of Oral Microbiology ACTA for microbial analysis. In addition, samples from 111 age-matched adult subjects without periodontitis were collected and analysed. Patients were >25 years old and samples were taken from sites with probing depths (PD) >6 mm (mean pockets depth = 6.97 ± 1.18 mm) that showed bleeding upon pocket probing. Patients had not used antibiotics in the past 3 months. The periodontally healthy controls were >25 years old and consecutive persons were invited to participate when they met the including criteria. Samples were taken from sites with PD < 4 mm, showing no bleeding on probing and no evidence of alveolar bone loss based on radiographic examination. All subjects were verbally informed about the purpose of the investigation and participated on the basis of written consent.

Sampling

Microbiological sampling in the patient group included selection of the deepest periodontal pocket in each quadrant of the dentition based on the probing depth measurements. In the healthy controls, all mesial and distal sites of all first molars from the buccal aspect were selected for microbiological sampling. Sample sites were isolated with cotton rolls and supragingival plaque was carefully removed with curettes and cotton pallets. Subsequently, two paper-points were inserted to the depth of the pocket and left in place for 10 s. Per subject, all paper points were pooled and transferred to a vial containing 1.5 ml of reduced transport medium (Syed et al. 1972). Upon arrival, samples were vortexed for 2 min. and split: 100 μl was used to prepare 10-fold serial dilutions in sterile phosphate buffered saline (PBS) for culturing, and 100 μl was used for RT PCR testing. The remaining 1.3 ml was stored at −20°C.

For anaerobic culture 100 μl of appropriate dilutions were plated on blood agar plates (Oxoid no. 2, Basingstoke, UK) which were supplemented with horse blood (5% v/v), hemin (5 mg/l) and menadione (1 mg/l) and incubated in 80% N_2, 10% H_2 and 10% CO_2, at 37°C for up to 14 days. *A. actinomycetemcomitans* was grown on trypticase soy-serum-bacitracin-vancomycin (TSBV) plates and incubated at 37°C in air +5% CO_2 for 3 days. Identification was done as described earlier (Boutaga et al. 2005).

Reference strains were grown as recommended by the American Type Culture Collection (ATCC). The strains used in this study were: *P. gingivalis* (W83), *T. forsythia* (clinical isolate), *A. actinomycetemcomitans* (NCTC 9710), *P. intermedia* (ATCC 25611), *M. micros* (clinical isolate), and *Fusobacterium polymorphum* (FDC 397).

Determination of the number of the total colony-forming units (CFU) per millilitre of the bacterial suspensions was performed by growing the bacteria 2–3 days in brain heart infusion (BHI) supplemented with 5 mg/l hemin and 1 mg/l menadione, and plating serial dilutions as described above (Boutaga et al. 2005).

Quantitative RT PCR

DNA isolation from plaque samples and bacterial reference cultures

From plaque samples and bacterial culture dilutions, 100 μl was used for automated DNA extraction and purification with the MagNA Pure DNA Isolation Kit III (Bacteria, Fungi) (Roche Molecular Diagnostics, Almere, the Netherlands). The protocol included 1-h pre-treatment with proteinase K (20 mg/ml) at 56°C. After isolation DNA was eluted in 100 μl elution buffer.

RT PCR

The primer/probe sets and PCR conditions were performed as described earlier (Boutaga et al. 2005). Briefly, RT-PCR amplification was performed in a total reaction mixture volume of 25 μl. The reaction mixtures contained 12.5 μl of 2 × TaqMan universal PCR master mix (PCR buffer, dNTP's, AmpliTaq Gold, reference signal [6-carboxy-X-rhodamine], uracil *N*-glycosylase, $MgCl_2$; Applied Biosystems, Foster City, CA, USA), 300–900 nM of the pathogen-specific primer, 50–100 nM pathogen-specific probe and 5 μl of purified DNA from plaque samples. The samples were subjected to an initial amplification cycle of 50°C for 2 min. and 95°C for 10 min., followed by 45 cycles at 95°C for 15 s and 60°C for 1 min. The data were analysed with ABI 7000 Sequence Detection System software (Applied Biosystems).

For quantification, the results from unknown plaque samples were projected on the counted pure culture standard curves of the target bacteria.

Possible inhibition of the RT-PCR was determined by comparing the results of spiked amplifications to the original samples. Each sample was spiked with 1000 CFU *Escherichia coli* DH5 (i.e. 50 CFU equivalents/PCR) before DNA isolation. In addition, negative amplifications were spiked with the target bacteria in numbers close to the threshold line. The primers and probe used for amplification of *E. coli* were described earlier (Huijsdens et al. 2002).

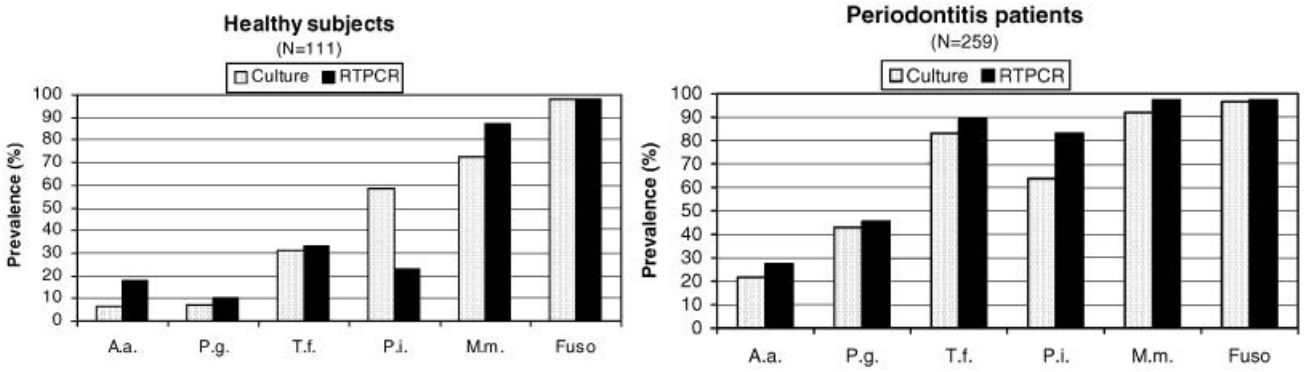

Fig. 1. Percentage of polymerase chain reaction and Culture positive samples for each test organism in subgingival plaque samples from periodontitis patients and periodontally healthy individuals. Aa, *A. actinomycetemcomitans*; Pg, *P. gingivalis*; Tf, *T. forsythia*; Pi, *P. Intermedia*; Mm, *M. micros*; Fuso., *Fusobacterium* spp.

Statistics

Data from both the anaerobic culture and RT-PCR were analysed for statistical significance by the two-tailed Fisher exact test or by the χ^2 test. p values <0.05 were considered statistically significant.

Results

Detection of pathogens with RT-PCR and anaerobic culture

A total of 370 subgingival plaque samples were analysed, including 259 samples from periodontitis patients and 111 samples from periodontally healthy individuals. Figure 1 shows the prevalence of target bacteria in periodontitis patients and periodontally healthy individuals by anaerobic culture and RT-PCR. In both groups more positive samples were detected by RT-PCR than by culture except for *P. intermedia*. Periodontal pathogens were more frequently detected in patients compared with healthy individuals. *A. actinomycetemcomitans* was the least frequently recovered species with both techniques in both groups (6.3% and 21.6% for culture and 18% and 27.4% for RT-PCR). The prevalence of *P. gingivalis* was low in healthy individuals, 7.2% by culture and 9.9% by RT-PCR and increased in periodontitis patients to 42.9% and 45.5%, respectively. The frequency of detection of *T. forsythia* with both techniques in healthy individuals showed no large differences, 31.4% and 33.2%, respectively, and had a prevalence of 83% by culture and 89.2% by RT-PCR in patients. *P. intermedia* showed a marked difference in the healthy individuals: 58.5% by culture and 23.2% by RT-PCR, while an increase in the periodontitis patients was observed to 63.7% by culture and 83% by RT-PCR. *M. micros* showed a high prevalence in healthy sites, 72.6% by culture and 87% by RT-PCR and in periodontal lesions, 91.9% by culture and 97.3% by RT-PCR. The most frequently detected species in both groups was *Fusobacterium* spp. (96.5% by culture and 97.3% by RT-PCR) in periodontitis patients and in healthy controls, 98.2% by culture and RT-PCR. The most prominent differences between RT-PCR and culture in both healthy controls and periodontitis patients were the culture-negative/PCR-positive samples (Fig. 2).

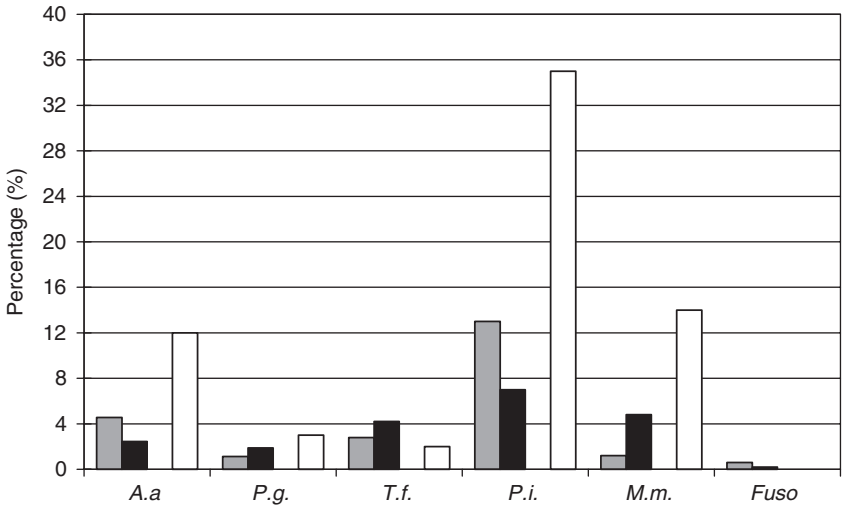

Fig. 2. The additional value of the real-time polymerase chain reaction (RT-PCR) for the six prominent periodontal pathogens in both periodontitis patients (grey and black bars) and healthy individuals (white bars). The *y*-axis represents percentage culture-negative/PCR-positive samples. The grey and black bars represent subgingival plaque samples with bacteria amounts $<10^4$ and $>10^4$ colony forming units (CFU), respectively. The white bars represent subgingival plaque samples with >10 CFU. De detection limit of anaerobic culture is 10 CFU in healthy individuals and 10^4 CFU in periodontitis patients.

Based on the number of positive samples in both groups odds ratios (ORs) were calculated (Table 1). For RT-PCR results, markers for disease were *P. gingivalis*, *T. forsythia*, *P. intermedia*, and *M. micros* (OR 8.6, 17.2, 9.6 and 5.2, respectively). *Fusobacterium* spp. showed a poor association with the disease with OR of 0.9 and 1.6 by culture and RT-PCR, respectively.

The highest ORs by culture were found for *P. gingivalis* and *T. forsythia*, 9.6 and 11.1, respectively. *A. actinomycetemcomitans* and *M. micros* revealed ORs of 4.1 and 4.2, respectively, and a significant difference ($p<0.05$) between the patients and the healthy individuals.

As indicated in Fig. 2, the cut-off value of culture in the periodontitis group is 10^4 CFU because of high amount of bacteria present in plaque. In the healthy individuals the cut-off

value is 10 CFU because of the low amount of bacteria present is plaque from this group. As a consequence the number of culture-negative/PCR-positive samples is the sum of the samples under the detection level of the culture and the RT-PCR results $>10^4$ CFU.

Quantitative detection of RT-PCR and anaerobic culture

Figure 3 shows the amount of bacteria obtained by culture and RT-PCR. Periodontitis individuals had significantly higher amount of all of the test species in the subgingival plaque samples compared with healthy subjects. In the periodontitis group CFU counts by RT-PCR were significantly higher ($p<0.05$) than numbers obtained by culture, except for *P. intermedia* and *Fusobacterium* spp. for which culture counts were significant higher ($p<0.05$). RT-PCR counts obtained from the healthy group were all higher than the CFU counts by culture for all the bacterial species tested ($p<0.05$).

Sensitivity and specificity

On the basis of the results described above, the sensitivity and specificity of the RT-PCR was calculated for the healthy controls with anaerobic culture as the reference method (Table 2). For the periodontitis patients, sensitivity and specificity were published in an other study by Boutaga et al. (2005).

The sensitivity for *A. actinomycetemcomitans*, *P. gingivalis*, *T. forsythia, M. micros* and *Fusobacterium* spp. when culture was considered as the reference standard was between 72% and 100%, with exception of *P. intermedia* (28%). The specificity for *A. actinomycetemcomitans*, *P. gingivalis*, *T. forsythia* and *P. intermedia* was between 83% and 97%. *Fusobacterium* spp. and *M. micros* showed specificity of 0% and 33%, respectively.

Table 1. Odds ratios (confidence interval-CI 95%) for each test microorganism in subgingival plaque samples from periodontitis patients and periodontally healthy individuals

Microorganism	Culture	RT-PCR
Actinobacillus actinomycetemcomitans	4.1 (1.8–9.3)*	1.7 (0.9–2.9)
Porphyromonas gingivalis	9.6 (4.5–20.6)*	8.6 (4.4–16.8)*
Tannerella forsythia	11.1 (6.6–18.6)*	17.2 (9.8–30.0)*
Prevotella intermedia/Prevotella nigrescens	1.2 (0.8–1.9)	9.7 (5.8–16.4)*
M. micros	4.2 (2.3–7.7)*	5.2 (2.0–13.3)*
Fusobacterium spp.	0.9 (0.2–4.9)	1.6 (0.2–9.5)

*$p<0.0001$.
RT-PCR, real-time polymerase chain reaction.

Discussion

A number of studies have evaluated the usefulness of detection and quantification of the bacterial DNA in plaque samples with different techniques (Savitt et al. 1988, 1990, Loesche 1992). These assays have limited specificity and sensitivity and are often laborious. Recently, RT-PCR assays have been developed for several oral bacterial pathogens (Boutaga et al. 2005), providing better detection tools for oral pathogens.

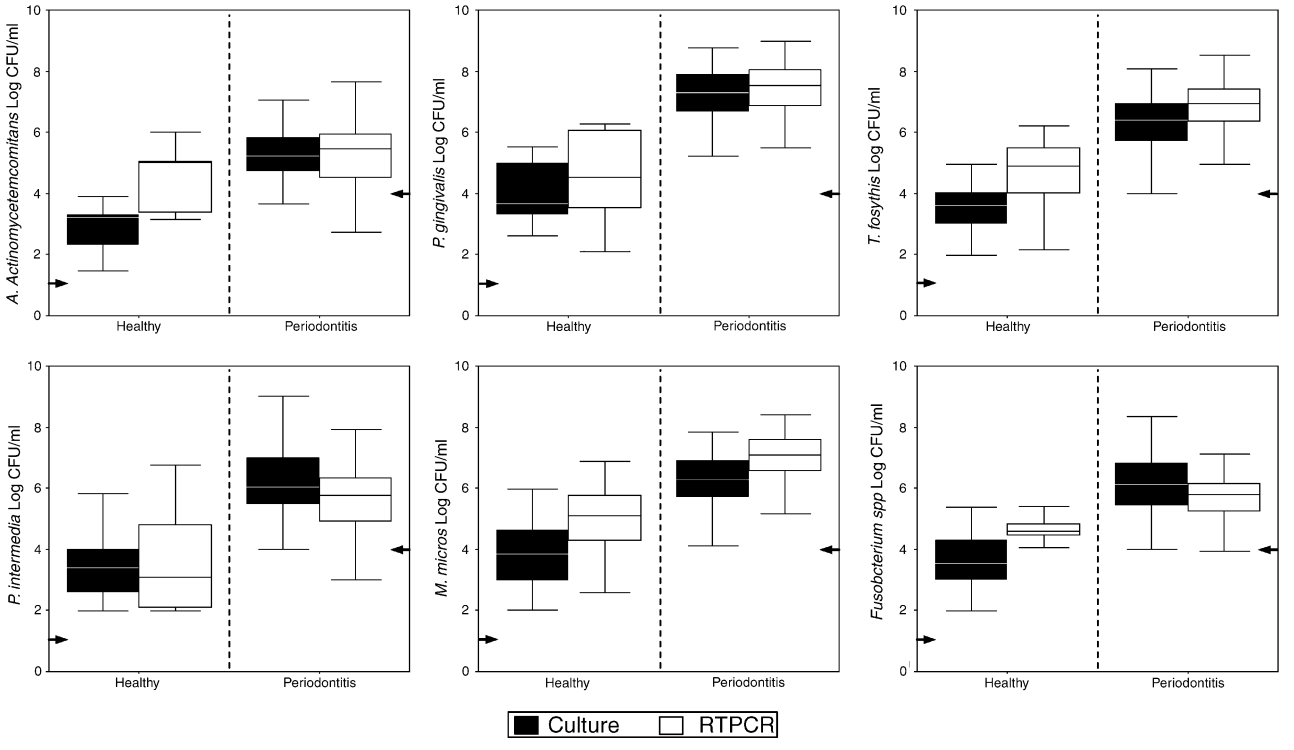

Fig. 3. Quantitative comparison (log transformed mean CFU/ml) was made for *A. actinomycetemcomitans*, *P. gingivalis*, *T. forsythia*, *P. intermedia*, *M. micros* and *Fusobacterium* spp. by anaerobic culture and real-time polymerase chain reaction (RT-PCR) in the culture-PCR positive samples in both periodontitis and healthy individuals. The *box* represents the first and third quartiles (rectangular boxes); the *line within the box* is the median. The arrows indicate the detection limit of the anaerobic culture.

Table 2. Sensitivity and specificity of *Actinobacillus actinomycetemcomitans* (Aa), *Porphyromonas gingivalis* (Pg), *Tannerella forsythia* (Tf), *Prevotella intermedia* (Pi), *Micromonas micros* (Mm) and *Fusobacterium* spp. (Fuso) in 111 healthy controls

Anaerobic culture and RT-PCR detected	No. of samples (%)				Sensitivity (%)	Specificity (%)
	RT-PCR (+)		RT-PCR (−)			
	culture (+)*	culture (−)	culture (+)	culture (−)		
Aa	5 (4.5)	15 (13.5)	2 (1.8)	89 (80.2)	72	86
Pg	8 (7.2)	3 (2.7)	0 (0)	100 (90.1)	100	97
Tf	29 (26.1)	8 (7.2)	6 (5.4)	68 (61.3)	83	89
Pi	18 (16.2)	8 (7.2)	47 (42.3)	38 (34.2)	28	83
Mm	77 (69.4)	20 (18)	4 (3.6)	10 (9)	95	33
Fuso	107 (96.4)	2 (1.8)	2 (1.8)	0 (0)	98	0

**Prevotella intermedia* and *Prevotella nigrescens*.
RT-PCR, real-time polymerase chain reaction.

In the present study, we have evaluated a RT-PCR for six oral bacterial pathogens in both periodontitis patients and healthy individuals in order to determine the additional value of this technique in samples with low numbers of pathogens, exploiting the excellent sensitivity of this technique in routine practice.

Both with the culture and the RT-PCR technique *P. gingivalis* and *T. forsythia* were prominent markers for periodontitis in adult patients. This is in agreement with other, culture-based studies (Van Winkelhoff et al. 2002). *P. intermedia* appeared to be strongly associated with periodontitis based by RT-PCR but not by culture. This difference is explained by the fact that *P. intermedia* and *Prevotella nigrescens* are not separated by culture identification whereas RT-PCR is specific for *P. intermedia*. *Fusobacterium* and *M. micros* are detected with high frequency in both healthy and periodontitis subjects in this study, suggesting their relationship with periodontitis is uncertain. The *Fusobacterium* genus consists of several (sub)species (Siqueira 2003) and the pathogenecity may vary depending on the (sub)species.

Remarkable is the low prevalance of *A. actinomycetemcomitans* and *P. gingivalis* in periodontal health, which supports the hypothesis that both species may represent exogenous oral pathogens (van Winkelhoff et al. 1997). With the exception of *P. intermedia* and *Fusobacterium* spp., all the bacteria were positively associated with periodontitis by culture with ORs ranging from 4.1 to 11.1. The association of *A. actinomycetemcomitans* with periodontitis was lost by RT-PCR. An explanation for this fact is that this species occurs in low numbers in periodontal lesions but can be detected by RT-PCR more frequently because of its high sensitivity.

Our findings also confirm observations of Griffen et al. (1998), who, using PCR technique, found an OR of 11.2 for *P. gingivalis* in adult patients with periodontitis. They found a healthy carrier rate for *P. gingivalis* of 25%, which is higher in comparison with the 9.9% found in our healthy group. This may be explained by the more strict clinical criteria we used for selection of periodontally healthy subjects in comparison with Griffen et al. (1998), where they accepted CAL measurements up to 5 mm and they also used more samples than we did.

If a putative pathogen can be detected frequently in healthy subjects, this suggests that not all humans are equally susceptible and/or that there is variation in virulence and pathogenic potential. It has been demonstrated, that the mean numbers of target bacteria were higher in periodontitis patients than in healthy controls (van Winkelhoff et al. 2002). As stated by Socransky et al. (1992) it is essential for the development of periodontitis that a pathogenic species is present, but still disease will not necessarily take place as long as the pathogen does not exceed a threshold for the host.

Recently, Jervoe-Storm et al. (2005) also compared RT PCR with conventional anaerobic cultivation. They investigated only 22 periodontitis patients for the presence of *A. actinomycetemcomitans, P. gingivalis, T. forsythia. P. intermedia* and *Fusobacterium nucleatum*. Our results (sensitivity and specificity) presented in this paper are in agreement and often higher than their data. They found lower specificity of (83.72%) for *P. gingivalis* and lower sensitivity of 66.67%, 94.29%, 92.16%, 33.33% and 73.02% for *A. actinomycetemcomitans*, *P. gingivalis*, *T. forsythia*, *P. intermedia* and *Fusobacterium nucleatum*. These discrepancies could be explained by low DNA isolation efficiency and it could also be because of inhibition of their PCR assays. Furthermore, in case of *F. nucleatum*, anaerobic culture is not able to distinguish between *Fusobacterium* species present in plaque samples. In contrast to this, we used in our study primer-probe combination amplifying 16 s rRNA gene from the *Fusobacterium* genus.

RT-PCR-positive/culture negative and RT-PCR-negative/culture positive discrepancies negatively influence the sensitivity and specificity of the RT-PCR for the tested oral bacteria. When culture was used as the golden reference standard, RT-PCR demonstrated a high sensitivity in the detection of *A. actinomycetemcomitans, P. gingivalis, T. forsythia. M. micro* and *Fusobacterium* spp. For *P. intermedia* the sensitivity was low, because of the RT-PCR negative-culture positive samples that also included *P. nigrescens*.

Nonnenmacher et al. (2004) tested five periodontal pathogens by RT-PCR, in periodontitis and healthy subjects. Our results were in agreement with their outcomes, both periodontitis patients and healthy controls were positive for the presence of target bacteria. However, median values in our study were higher in samples from periodontitis patients comparing with their results. The data for *A. actinomycetemcomitans, P. gingivalis, P. intermedia* and *M. micros* ranges between 10^3 and 10^8 CFU/ml (this study) *versus* $<10^2$ and 7.310^3 CFU/ml in periodontitis and healthy subjects, respectively. Furthermore the authors also discussed the application of eubacterial primers to detect all the bacterial species tested, however no data were shown.

Lau et al. (2004) also validated a RT PCR assay for identification and quantification of *A. actinomycetemcomitans, P. gingivalis* and *T. forsythia* from subgingival plaque taken from subjects with different periodontal conditions. *P. gingvalis* was detected in 81.3% of periodontitis subjects by RT-PCR and in 84.4% by culture. These results are much higher than our results, 42.9% by culture and 45.5% by RT-PCR. *T. forsythia* was detected in 73.7% by RT-PCR in the healthy controls, while in our study the bacterium was detected

in 33.2%. Another major difference was the low prevalence of *T. fosythia* in periodontitis subjects by RT-PCR (25% *versus* 89.2% in our study). The discrepancies could be because of the primers and probes used, which are based on a single copy gene while we used 16s rRNA gene as a target gene. The high sensitivity and specificity of the RT-PCR detection of *P. gingivalis* shown in our study is periodontitis patients and healthy controls is clearly better than the results obtained by RT-PCR technology seems much more rapid than all oral bacteria assays previously described. In addition, the analytical sensitivity of the assay is excellent, as the detection limit obtained with spiked samples was 1–50 CFU and the detection limit of the anaerobic culture is 10^4 CFU/ml in periodontitis patients. This explains the discrepancies between culture and PCR in both periodontitis and healthy individuals. However, the bacteria could not be recovered from some clinical specimens in periodontitis patients and healthy individuals even when the load was higher than 10^4 CFU/ml by PCR. This may be because of limitations of the culture with respect to detecting non-viable bacteria, the difficulty in recovering even cultivable species when they are found in low numbers, the inability of some species to grow reliably on selective media and high load of bacteria in clinical specimen may hamper the detection of the target bacteria. The TaqMan PCR assay, using DNA as a template, will detect both living and dead bacteria, whereas culture will detect only living bacteria. Although this later may influence the quantitative results, there is a high correlation between culture and RT-PCR (Boutaga et al. 2003, 2005). As such the presence of dead bacteria may increase the sensitivity of the RT-PCR by the increased amount of templates present in clinical samples. This might be an advantage when low amount of bacteria are present in example small samples, sputa or blood.

In summary, the results of our study show that major periodontal pathogens can reliably be detected by both the culture and the RT-PCR technique. Despite the higher sensitivity and specificity of the PCR technique, the results showed an excellent agreement with conventional culture except for *A. actinomycetemcomitans*. The most prominent advantages of the RT-PCR technique are the high sensitivity and specificity of the technique and the possibility of rapid and cost-effective quantitative detection of these oral pathogens. This new molecular approach may benefit clinical microbiology for diagnosis of both oral and non-oral infectious diseases.

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Address:
Paul H. M. Savelkoul
Department of Medical Microbiology and Infection Control
VU University Medical Center
PO Box 7057, 1007 MB Amsterdam
The Netherlands
E-mail: p.savelkoul@vumc.nl

Clinical Relevance

Scientific rationale for the study: Molecular microbiological techniques have been introduced to detect and quantify periodontal pathogens. These new approaches such as the RT-PCR need to be validated and compared with the anaerobic culture technique in both periodontal health and disease. Data on the surplus value of RT-PCR technique are not currently available for the major periodontal pathogens.

Principal findings: With RT-PCR more patients and healthy controls were positive for all six pathogens tested. Both techniques identified *P. gingivalis*, *T. forsythia* and *M. micros* as significant markers for periodontitis. *P. intermedia* appeared a significant marker for disease by RT-PCR only. For most pathogens, the difference in prevalence by both techniques was not greater than 7%, except for *P. intermedia*.

Practical implications: The study results show that both techniques are valuable tools to quantitatively detect periodontal pathogens. The RT-PCR allows for detection of smaller number of pathogens but the clinical relevance of this observation has not been established. The great advantage of the RT-PCR is the fact that it does not require viable bacterial cells for detection.