Discrepancy between culture and DNA probe analysis for the detection of periodontal bacteria


Abstract. The purpose of this study was to compare a commercially available DNA probe technique with conventional cultural techniques for the detection of Actinobacillus actinomycetemcomitans, Porphyromonas gingivalis and Prevotella intermedia in subgingival plaque samples. Samples from 20 patients with moderate to severe periodontitis were evaluated at baseline and during a 15 months period of periodontal treatment. Paperpoints from 4 periodontal pockets per patient were forwarded to Omnigen for DNA probe analysis, and simultaneously inserted paperpoints from the same pockets were analyzed by standard culture techniques. In addition, mixed bacterial samples were constructed harbouring known proportions of 25 strains of A. actinomycetemcomitans, P. gingivalis and P. intermedia each. A relatively low concordance was found between both methods. At baseline a higher detection frequency was found for A. actinomycetemcomitans and P. gingivalis for the DNA probe technique; for P. intermedia the detection frequency by culture was higher. For A. actinomycetemcomitans, 21% of the culture positive samples was positive with the DNA probe. Testing the constructed bacterial samples with the DNA probe method resulted in about 16% false positive results for the 3 species tested. Furthermore, 40% of P. gingivalis strains were not detected by the DNA probe. The present data suggest that at least part of the discrepancies found between the DNA probe technique used and cultural methods are caused by false positive and false negative DNA probe results. Therefore, the value of this DNA probe method for the detection of periodontal pathogens is questionable.

Several bacteria, including Actinobacillus actinomycetemcomitans, Porphyromonas gingivalis, Prevotella intermedia, Peptostreptococcus micros, Campylobacter rectus, Fusobacterium nucleatum and spirochaetes, are often isolated in relatively high proportions from deep pockets of patients with periodontitis. For a long period of time, culture techniques have been generally used for the detection of these bacteria. More recently, other techniques have become available, based on the detection of DNA or antigens of the bacteria to be studied (Listgarten 1992). A method, which is commercially available as DMDx (Omnigene, Cambridge, MA, USA), is based on either whole chromosomal DNA probes, for the detection of P. gingivalis and P. intermedia, or on cloned DNA fragments, for the detection of A. actinomycetemcomitans (French et al. 1986, Strzempek et al. 1987). Although the presence of suspected periodontal pathogens detected by this method has been correlated with periodontitis (Savitt et al. 1988), information on the correlation of this detection method with culture techniques for samples from periodontitis patients is limited (Loesche et al. 1992), and as far as we know not available for samples from patients during treatment.

The purpose of the present study was to compare the DMDx method with culture techniques for the detection of A. actinomycetemcomitans, P. gingivalis and P. intermedia in subgingival samples from periodontitis patients and to test the DMDx method for constructed samples of pure bacterial cultures.
Material and Methods

Clinical procedures
The microbiological data in this study are derived from a treatment study with subgingival application of a minocycline gel (Timmerman et al. 1996). In short, 20 patients (7 male and 13 female), aged 39–59 years, were selected on the basis of at least one periodontal pocket of ≥5 mm in association with interproximal loss of attachment of ≥3 mm in at least one site in each quadrant. At least 3 natural teeth should be present in each quadrant. Patients who had received antibiotics 3 months prior to the study were excluded. At baseline all patients received oral hygiene instruction, and scaling and root planing on all teeth. Pockets were treated by administering a 2% minocycline-gel (Ledercer) or a placebo vehicle by filling each pocket up to the gingival margin. At the various time intervals during and after treatment, no significant differences were found in detection rate of the various bacteria for the minocycline group compared to the placebo group (Timmerman et al. 1996). Therefore, in the present study the data were considered to be derived from one group.

Clinical samples
At baseline, 3, 6, 9, 12, and 15 months 2 paper points were simultaneously inserted into the 4 pockets with the greatest amount of loss of attachment in each quadrant. During the sampling procedure, the two paper points were kept together in such a way, that the points were in contact over their full length, this to ensure that the two paper points could be regarded as identical. 1 paperpoint from each pocket was forwarded to Omnigene for determination of the levels of P. gingivalis, P. intermedia, A. actinomycetemcomitans, C. rectus and F. nucleatum by DNA probe technology. The data obtained for these 4 pockets were pooled.

The other 4 paper points were pooled in 0.9 ml reduced transport fluid (RTF; Syed & Loesche 1972). The samples were processed within 2 h. Samples were diluted in RTF and plated on the following media: (1) 5% horse blood agar plates incubated anaerobically (10% H2, 10% CO2, 80% N2) for a maximum of 14 days for the detection of P. gingivalis, P. intermedia, and total anaerobic cultivable flora; (2) TSBV medium (Slots 1982) incubated in air +5% CO2 for the detection of A. actinomycetemcomitans. P. gingivalis and P. intermedia were identified on the basis of colony morphology, indole production, glucose fermentation, hemagglutination, trypsin-like activity, and the reaction pattern on ATB 32A (Biomerieux). A. actinomycetemcomitans was identified on the basis of colony morphology, catalase reaction, growth on the selective medium, and if necessary the presence of enzymatic activity as determined by the API ZYM kit (Biomerieux).

Constructed samples
In addition to the clinical samples a number of samples was constructed with a known composition of the 3 test bacteria. 25 fresh clinical isolates of A. actinomycetemcomitans, 25 of P. gingivalis and 25 isolates of P. intermedia were used. These strains were streaked to purity on blood agar plates, and identified using the standard techniques as described above. Bacteria were cultured on blood agar plates for 2–6 days. Suspensions in RTF were made to an OD650 of 0.1, which corresponds to approximately 2×108 colony forming units (cfu) per ml. For each species in total 25 positive samples and 28 negative samples were constructed. 13 mixtures were made of A. actinomycetemcomitans and P. gingivalis, and 12 of A. actinomycetemcomitans and P. intermedia. Furthermore, 12 pure cultures of P. gingivalis and 13 of P. intermedia were used. 3 samples were prepared containing a mixture of 9 oral bacteria excluding the 3 test bacteria. A droplet of 5 μl of the constructed sample (±1×106 cfu) was put on a hydrophobic parafilm surface, and allowed to adsorb completely on a paper point. The paper point was forwarded to Omnigene for quantitative DNA probe analysis in the same way as the samples from the patients.

Results

Clinical samples
At baseline, 3 out of 20 patients were culture positive for A. actinomycetemcomitans, 14 for P. gingivalis and 16 for P. intermedia (Table 1). With the DNA probe technology, the detection rate was significantly higher: 10 patients were positive for A. actinomycetemcomitans, all 20 for P. gingivalis and 18 for P. intermedia. The % of culture positive samples for A. actinomycetemcomitans and P. intermedia remained

| Table 1. Comparison of the detection of A. actinomycetemcomitans (Aa), P. gingivalis (Pg) and P. intermedia (Pi) by culture and by a DNA probe technique for subgingival samples from 26 periodontitis patients; each individual was sampled once at baseline and 5 times during treatment for 15 months |
|-----------------|--------|-------|-------|-------|-------|-------|
|                 | Aa+    | Aa−    | Pg+    | Pg−    | Pi+    | Pi−    |
| baseline culture | + 2 1 | 14 0 | 16 0 | 8 9 | 6 0 | 2 2 |
| 3 month culture  | + 0 3 | 5 5 | 12 6 | 1 16 | 4 6 | 0 2 |
| 6 month culture  | + 0 1 | 11 1 | 11 4 | 2 17 | 4 4 | 2 3 |
| 9 month culture  | + 0 2 | 6 4 | 6 9 | 3 15 | 6 4 | 0 5 |
| 12 month culture | + 1 2 | 5 5 | 6 12 | 2 15 | 2 8 | 0 2 |
| 15 month culture | + 0 2 | 4 3 | 7 10 | 2 16 | 5 8 | 0 3 |
| total during treatment culture | + 1 10 | 31 18 | 42 41 | 10 79 | 21 30 | 2 15 |
Table 2. Detection of A. actinomycetemcomitans (Aa), P. gingivalis (Pg), and P. intermedia (Pi) in subgingival samples by a DNA probe technique in relation to the numbers of bacteria detected by culture.

<table>
<thead>
<tr>
<th>Culture bacterial numbers</th>
<th>Baseline</th>
<th>During treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>number of samples</td>
<td>number of DNA positive samples</td>
</tr>
<tr>
<td>Aa</td>
<td>cfu&gt;6000</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>cfu&lt;6000</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>17</td>
</tr>
<tr>
<td>Pg</td>
<td>cfu&gt;6000</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>cfu&lt;6000</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>Pi</td>
<td>cfu&gt;6000</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>cfu&lt;6000</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>4</td>
</tr>
</tbody>
</table>

approximately the same during treatment, whereas the % of culture positive samples for P. gingivalis decreased from 70% at baseline to 50-35% at the 3 to 15 months intervals. However, using the DNA probe the percentage of A. actinomycetemcomitans and P. gingivalis positive samples also decreased. During treatment, culture positive samples were often negative in the DNA probe assay: for A. actinomycetemcomitans in 91% of the samples, for P. gingivalis 37%, and for P. intermedia in 49% of the samples during treatment.

As the DNA probe technique produces positive results if bacteria are present in quantities of ≥6000 cfu, the detection frequency in the clinical samples was calculated in relation to the amount of bacteria cultured (Table 2). For A. actinomycetemcomitans, no correlation was found between the detection rate by the DNA probe and the numbers of bacteria cultured. For P. gingivalis and P. intermedia the detection rate of the DNA probe assay was higher in samples containing ≥6000 cfu compared to samples with <6000 cfu.

Table 3. Detection of A. actinomycetemcomitans (Aa), P. gingivalis (Pg), and P. intermedia (Pi) by a DNA technique for in vitro constructed samples.

<table>
<thead>
<tr>
<th>DNA probe analysis</th>
<th>Aa+</th>
<th>Aa-</th>
<th>Pg+</th>
<th>Pg-</th>
<th>Pi+</th>
<th>Pi-</th>
</tr>
</thead>
<tbody>
<tr>
<td>presence in sample</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>24</td>
<td>1</td>
<td>15</td>
<td>10</td>
<td>24</td>
<td>1</td>
</tr>
<tr>
<td>-</td>
<td>4</td>
<td>24</td>
<td>5</td>
<td>23</td>
<td>5</td>
<td>23</td>
</tr>
</tbody>
</table>

Table 4. Specificity and sensitivity of a DNA probe technique for the detection of A. actinomycetemcomitans (Aa), P. gingivalis (Pg), and P. intermedia (Pi) in samples from periodontitis patients, in constructed samples, and in clinical samples harbouring ≥6000 cfu of the particular species.

<table>
<thead>
<tr>
<th>Constructed samples (%)</th>
<th>Clinical samples at baseline (%)</th>
<th>Clinical samples during treatment (%)</th>
<th>Clinical samples ≥6000 cfu (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aa sensitivity</td>
<td>96</td>
<td>67</td>
<td>9</td>
</tr>
<tr>
<td>Aa specificity</td>
<td>86</td>
<td>53</td>
<td>89</td>
</tr>
<tr>
<td>Pg sensitivity</td>
<td>60</td>
<td>100</td>
<td>63</td>
</tr>
<tr>
<td>Pg specificity</td>
<td>82</td>
<td>0</td>
<td>59</td>
</tr>
<tr>
<td>Pi sensitivity</td>
<td>96</td>
<td>100</td>
<td>51</td>
</tr>
<tr>
<td>Pi specificity</td>
<td>82</td>
<td>50</td>
<td>88</td>
</tr>
</tbody>
</table>

Specificity and sensitivity

On the basis of the results obtained, the specificity and sensitivity of the DNA probe method was calculated with the culture technique as a reference (Table 4). The specificity of the DNA based method varied between 82 and 86% for the constructed samples; the sensitivity for A. actinomycetemcomitans and P. intermedia was high, but amounted only 60% for P. gingivalis. For the clinical samples at baseline, the sensitivity ranged from 67 to 100%, however, the specificity was low, ranging from 0-50%. During treatment, the sensitivity was considerably lower, especially for A. actinomycetemcomitans; however, the specificity was higher compared to that at baseline. If only clinical samples containing ≥6000 cfu were considered, the sensitivity remained low for A. actinomycetemcomitans.

Discussion

The DNA probes as used in the present study have been evaluated by French et al. (1986) and Strzempek et al. (1987) for pure cultures and constructed mixed samples. They found all 2-4 strains tested of P. gingivalis and P. intermedia to be positive with a whole genomic probe. A low level of cross-reactivity (<1%) was found for a limited number of other species. A whole genomic probe of A. actinomycetemcomitans has been found to be cross-reactive, especially with Haemophilus bacteria, and is therefore considered to be not useful for clinical samples (French et al. 1986, Strzempek et al. 1987). In contrast, a probe based on a cloned fragment of A. actinomycetemcomitans is not cross-reactive (French et al. 1986). Savitt et al. (1988) compared DNA probes with culture techniques for samples from peri-
odontal pockets and found a much higher detection rate with the DNA probe compared to culture. This may be partly explained by a relatively high detection level of the culture technique used, as the bacteria were only counted on plates with less than 200 colonies; this implicates that levels of <0.5% have not been detected. Loesche et al. (1992) compared culture technology with the DNA probe technique and two immunological techniques to identify among others Actinobacillus actinomycetemcomitans and P. gingivalis in samples from periodontal pockets. They observed a higher detection rate for P. gingivalis with the DNA probe as compared to culture, and approximately the same detection rate for A. actinomycetemcomitans. However, in their study the concordance between both methods is relatively low. Often the 3 non-cultural methods show the presence of the bacterium, but the culture was negative. Therefore, Loesche et al. (1992) concluded that culture techniques probably underestimate the presence of Actinobacillus actinomycetemcomitans and P. gingivalis. On the other hand, false positive reactions of the DNA technique and the immunological methods used can not be excluded.

In the present study, a considerable discrepancy was found between culture and DNA probe technology for samples from periodontal pockets. In order to gain more insight in possible drawbacks, bacterial samples were constructed of pure and mixed cultures, which were treated in the same way as the clinical samples. For A. actinomycetemcomitans and P. intermedia, the specificity of the DNA method was comparable for the clinical samples and the constructed samples. Therefore, samples positive in the DNA test but negative by culture were probably false positive DNA results, caused by cross-reactivity of the probe. If a cross-reactivity of 0.1% is assumed (Strzempko et al. 1987), the presence of 6,000,000 cfu of the specific bacterium, which is the detection limit for the DNA test, the DNA method showed a high sensitivity. However, for P. gingivalis only 60% of the strains tested were identified by the probe. Whether this represents a specific subset of P. gingivalis strains is not known. In the past few P. gingivalis and P. intermedia strains have been evaluated (French et al. 1986, Strzempko et al. 1987). A minimum detection limit of 10 cfu can theoretically be obtained for culture techniques, i.e. one colony growing on a plate to which 100 μl of the undiluted sampled is applied. For A. actinomycetemcomitans, we were often able to detect 10–100 cfu per sample, most likely due to the use of a selective medium. Despite the use of a non-selective medium low numbers of P. intermedia and P. gingivalis could be detected by carefully searching for black to brown pigmented colonies with a stereomicroscope. These 2 species were found in amounts of <100 cfu per sample, which corresponded to <0.01% of the total cultivable flora. For clinical samples containing ≥6000 cfu of the specific bacterium, which is the detection limit for the DNA test, the DNA method showed a high sensitivity for P. gingivalis, and a moderate sensitivity for P. intermedia. A. actinomycetemcomitans was cultured in amounts above the detection limit for the DNA probe in 5 samples, but detected by this probe in only one sample, resulting in a very low sensitivity of 20%. Whether the higher detection frequency of the culture technique has clinical significance remains to be established, as it is not clear to which extent low levels of these bacteria contribute to the disease. On the other hand, at baseline the detection frequency of A. actinomycetemcomitans and P. gingivalis with the DNA probe was higher than by culture. This may be explained by the presence of non-viable bacteria in the sample or to the processing technique. It is unlikely that the time interval between sampling and processing of maximally 2 h was of importance, as we found previously no reduction in the levels of A. actinomycetemcomitans, P. gingivalis and P. intermedia that can be cultured from subgingival samples for up to 4 h storage in RTF (Van Steenbergen et al. 1993). For A. actinomycetemcomitans and P. gingivalis, most of the DNA positive samples were obtained from subjects who were negative by culture at all examinations (for A. actinomycetemcomitans, 15 out of 18 samples and for P. gingivalis 23 out of 27 samples). Thus, the higher detection frequency of these species by the DNA test could not be explained by a low detection frequency by culture in subjects who were positive for the bacteria in at least one occasion. During treatment, a considerably higher percentage of culture positive samples was negative in the DNA test as compared to baseline. The low numbers of test bacteria present after treatment in the relatively shallow pockets may contribute to this phenomenon. Alternatively, part of this difference may be caused by a non-specific DNA test at baseline due to cross-reactivity with the large number of other bacteria that are present before treatment.

In conclusion, considerable differences were observed between the DMDx DNA probe technique and cultural methods for the detection of periodontal bacteria in a clinical setting. Results from constructed samples indicate that, especially for P. gingivalis, the DNA probe assay can give false negative data. Therefore, the value of this DNA probe methodology for the detection of periodontal bacteria is questionable.

Zusammenfassung
Discrepanz zwischen kultureller und DNA-Sonden-Analyse beim Nachweis von parodontalen Bakterien
par analyse avec sonde ADN
paris ginvialis et P. intermedia ent­
hielten. Zwischen beiden Methoden wur­
die Nachweisgenauigkeit der Kultur­
tehnik besonders, für A. actinomycetemcomitans und P.
ginvialis vorgefunden. Für P. intermedia war
was die Nachweisgenauigkeit der Kultivierungs­
tehnik höher, für A. actinomycetemcomitans
zeigten 21% der Kultur-positiven Plaquepro­
en einen positiven DNA-Sondentest. Der
Test mit den künstlichen Bakterienproben er­
gab für die DNA-Methode ungefähr 16% falsch positive Ergebnisse bei den 3 Testspe­
zies. Des weiteren wurden 40% der P. ginvialis-Stämme nicht mit der SNA-Sonde nachgewiesen. Die vorliegenden Daten lassen annehmen, daß wenigstens ein Teil der Dis­
dizis in der DNA-Sondentest mit der Anzüchtungs methode gefunden wer­
den, von falsch positiven und falsch negati­
denen Ergebnissen der DNA-Sondenverüf­
acht werden. Daher ist der Wert dieser
DNA-Sondennachweise für die Parodontopathogenen fraglich.

Résumé
Contradiction entre les résultats de la recher­
che des bactéries parodontales par culture et par analyse avec sonde ADN
Le but de cette étude était de comparer une
méthode commercialisée utilisant la sonde
ADN avec des méthodes classiques par culture pour la recherche d’Actinobacillus actinomycetemcomitans, de Porphyromonas gingivalis et de Prevotella intermedia dans les échantillons de plaque sous-gingivale. Des échantillons prélevés chez 20 patients atteints de parodontite modérée à sévère ont été évalués au début (baseline) et pendant une période de traitement parodontal de 15 mois. Les pointes de papier de 4 poches pa­
donaldales par patient ont été adressées à
Ommeghe pour analyse par sonde ADN, et des pointes de papier introduites en même
temps dans les mêmes poches ont été analy­
sées par techniques de culture ordinaires.

De plus, on a construit des échantillons bactériens mixtes contenant des propor­
cions connues de 25 souches d’A. actino­
mycetemcomitans, P. gingivalis et P. inter­
media. La concordance trouvée entre les 2 méthodes était relativement basse. Au début,
on a trouvée une fréquence de détection
de A. actinomycetemcomitans et de P. gingi­
valis plus élevée avec la technique par sonde
ADN; pour P. intermedia, la fréquence de
détection par culture était plus élevée. Pour
A. actinomycetemcomitans, 21% des échantil­
lon positifs en culture étaient positifs avec la sonde ADN. Avec la méthode par
sonde ADN, le test des échantillons bacté­
riens construits donnait environ 16% de ré­
sultats faux positifs pour les 3 espèces
considérées. De plus, 40% des souches de P.
gingivalis n’étaient pas mises en évidence
par sonde ADN. Ces données semblent indi­
cquer qu’il y a un certain consensus entre les
récisions trouvées entre la méthode par sonde
ADN et les méthodes par culture sont
causées par des résultats faux positifs et faux négatifs par sonde ADN. La méthode
de recherche des pathogènes parodontaux
par sonde ADN est donc d’une valeur dou­
teuse.

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