Quantitative Analysis of *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis* in Adolescents from Ghana

Frida Söderqvist and Nina Willdén

Tutors: Anders Johansson, Rolf Claesson, Carola Höglund Åberg
ABSTRACT
Periodontitis is an inflammatory disease associated with different bacterial species, including *P. gingivalis* and *A. actinomycetemcomitans*. This study is based on a previous study where bacterial samples were collected from periodontal pockets on adolescents in Ghana. The objective was to quantitatively analyse the samples concerning *A. actinomycetemcomitans* and *P. gingivalis* and to correlate them towards each other. Furthermore, *A. actinomycetemcomitans* was correlated against development of attachment loss. Out of 315 available samples from periodontally healthy adolescents, 162 were successfully analysed using qPCR. Excel and SPSS were used for statistical calculations and analyses. Medium levels (1 – 10 %) of *A. actinomycetemcomitans* indicated a significant higher risk (p = 0.005) for development of attachment loss compared to low levels (< 1 %). Also, a significant risk (p = 0.024) for development of attachment loss was found for levels over 1 % compared to levels lower than 1 % (including negative samples). Surprisingly, no significant higher risk was found when comparing *A. actinomycetemcomitans* negative samples with low, medium and high (> 10 %) bacterial proportions. No significant correlation (p = 0.144) was found between proportions of *A. actinomycetemcomitans* and *P. gingivalis* in the same sample. The concluding remarks are that *A. actinomycetemcomitans* detected in levels exceeding 1 %, particularly 1 – 10 %, are most associated with development of attachment loss. There is no significant negative correlation between high levels of *A. actinomycetemcomitans* and *P. gingivalis* in periodontal pockets before attachment loss can be detected.
INTRODUCTION

Periodontitis is an inflammatory disease affecting the hard and soft tissues supporting the teeth. The inflammation is caused by different bacteria which colonizes the tooth surface in a biofilm (Darveau 2010). Unless removed, this process eventually leads to destruction of the connective tissue and alveolar bone. The destructive process is seen clinically as increased clinical attachment loss (CAL), a measure of bone loss between the cementoenamel junction and the bottom of the pocket. Without proper treatment, the disease may result in loss of teeth. As an individual goes from periodontal health to disease, the dental biofilm shifts from a dominant gram-positive flora to a gram-negative. One well-known gram-negative bacteria associated with periodontal disease is *Porphyromonas gingivalis*. Another important periodontal pathogen is *Aggregatibacter actinomycetemcomitans*, which is a facultative, gram-negative rod. The latter of the species has been associated to localized aggressive periodontitis (Darveau 2010; Henderson *et al.*, 2010). Early onset of disease, rapid progression of attachment loss and destructions of the periodontium visible both radiographically and clinically constitutes some of the diagnostic criteria for aggressive periodontitis (Albandar 2014).

Two different toxins are produced by *A. actinomycetemcomitans*, a cytolethal distending toxin and a leukotoxin (Henderson *et al.*, 2010). *A. actinomycetemcomitans* includes six different serotypes (a-f). Within serotype b there is a highly virulent genotype known as the JP2 clone (Haubek and Johansson 2014). The JP2 clone is missing a 530 base pair sequence within the promoter region of the leukotoxin operon. The genotype having this deletion is highly virulent since it produces high levels of leukotoxin (Brogan *et al.*, 1994). It has been found that individuals harbouring the JP2 clone of *A. actinomycetemcomitans* are at increased risk for developing periodontal attachment loss compared to carriers of other *A. actinomycetemcomitans* genotypes (Haubek *et al.*, 2004; 2008; Höglund Åberg *et al.*, 2014). In Moroccan adolescents diagnosed with aggressive periodontitis the JP2 clone of *A. actinomycetemcomitans* is found only in small proportions, even though it is considered to be an etiological factor (Rylev *et al.*, 2011). This finding indicates that presence rather than amount of the JP2 clone might be an important factor in the development of periodontitis.
A previous study on adolescents in Ghana indicates a strong association between presence of \textit{A. actinomycetemcomitans}, in particular the highly-leukotoxic JP2 genotype, and an increased risk for developing periodontal attachment loss (Höglund Åberg \textit{et al.}, 2014). At renewed sampling after a 2-year follow-up period, the data shows a relatively low presence of \textit{A. actinomycetemcomitans}, in comparison to other species, in the deep pockets (Dahlén \textit{et al.}, 2014). This suggest that \textit{A. actinomycetemcomitans} has an important role at the initiating stage of the attachment loss, before pathological periodontal pockets can be detected clinically. However, as the pocket deepens this bacterium is assumed to be replaced by gram-negative obligate anaerobe species such as \textit{P. gingivalis} (Dahlén \textit{et al.}, 2014; Höglund Åberg \textit{et al.}, 2014; 2015).

Studies have shown that \textit{P. gingivalis} has properties which enable it to inhibit the activity of the leukotoxin produced by \textit{A. actinomycetemcomitans} in vitro (Johansson \textit{et al.}, 2000). Moreover, \textit{P. gingivalis} strains with high proteolytic capacity and elevated formation of biofilm, has the ability to de-attach and outcompete \textit{A. actinomycetemcomitans} in microbial biofilm. A virulent factor produced by \textit{P. gingivalis} can disrupt the formation of biofilm by \textit{A. actinomycetemcomitans} and also de-attach a pre-formed \textit{A. actinomycetemcomitans} biofilm (Takahasi \textit{et al.}, 2013; Haraguchi \textit{et al.}, 2014). These interactions could affect the composition of the biofilm present in different stages of disease initiation and progression.

In the previous studies no quantitative analysis has been done regarding \textit{A. actinomycetemcomitans} and its potential role in initiation and progression of periodontal disease. The aim of this study was to quantitatively analyse \textit{A. actinomycetemcomitans} and \textit{P. gingivalis} in periodontal samples collected at base line in a longitudinal study on adolescents in Ghana (Höglund Åberg \textit{et al.}, 2012). In each sample, the proportions of \textit{A. actinomycetemcomitans} was correlated to the proportion of \textit{P. gingivalis}, as well as against any attachment loss (CAL ≥ 3 mm) developed during a 2-year follow-up period. The samples was analysed by the use of quantitative PCR (qPCR) to obtain total bacterial amounts and proportions of \textit{A. actinomycetemcomitans}, the JP2 genotype and \textit{P. gingivalis}. Also, the qPCR method will be compared to the
conventional PCR used in the earlier study regarding detection of *A. actinomycetemcomitans* (Höglund Åberg *et al.*, 2012).

The first hypothesis of this study is that attachment loss related to the presence of *A. actinomycetemcomitans* may in turn not be related to the amount of *A. actinomycetemcomitans* detected in the individual before clinical symptoms appear. The second hypothesis is that there is a negative correlation between the amount of *A. actinomycetemcomitans* and *P. gingivalis* in the same sample.

Data obtained from quantitative analyses of *A. actinomycetemcomitans* and *P. gingivalis* present in individuals examined clinically, will provide increased understanding concerning the role of different bacteria during the development of periodontal attachment loss. This knowledge could be of value for progress of specific and effective prevention strategies.

**MATERIALS AND METHODS**

**Literature search**

A number of articles were handed to us by our tutors. Additional searches were done at the PubMed library using the following MESH-terms: periodontitis, etiology, microbiology, bacteria, *Aggregatibacter actinomycetemcomitans*, JP2, *Porphyromonas gingivalis*, correlation and detachment. The terms were used in different combinations in order to obtain a sufficient amount of relevant articles. The initial searches resulted in 321 articles, of which 25 were selected as interesting. Based on the contents of the abstracts, 14 articles were considered relevant for this study. Among the selected articles some references of interest were found. These articles were searched on manually at PubMed.

**Study population and microbial samples**

The present study is based on clinical and microbial material available from previous studies on periodontitis, the first of which was initiated in 2009. The material consists of microbial samples collected from first molar subgingival pockets in 500 Ghanaian
adolescents aged 10 to 19 years (mean age 13.2 ± 1.5 years). The samples were analysed by culturing and conventional PCR. At 2-year follow-up the individuals were re-examined clinically. The presence of *A. actinomycetemcomitans* and its characteristics were correlated to the risk of developing periodontal attachment loss (Höglund Åberg *et al*., 2014). The samples were frozen and stored for further studies in the laboratory. In this study the same samples were re-used for quantitative analysis. Detailed information concerning study population and sampling are to be found in previous publications (Höglund Åberg *et al*., 2012).

**Inclusion criteria**

Since the intention was to predict risk for periodontal disease, only samples from individuals healthy at base line were included. Participants lacking attachment loss (CAL < 3 mm) at base line and accessible at the 2-year follow-up were included in the study (Figure 1). Samples containing a total amount of ≥ 10.000 bacteria/µl were included. Concerning specific species (*P. gingivalis*, *A. actinomycetemcomitans*) and genotypes (JP2 clone), the cut off value was set to ≥ 100 bacteria/µl. A total of 162 samples were successfully analysed.

**Ethical considerations**

Ethical permits are issued for usage of the samples collected from the Ghanaian adolescents in research purposes (Ghana, IRB nr 000 1276 and Umeå University Dnr 2010-188-31M). Signed consents from the parents or guardians of the Ghanaian adolescents are documented. They have been informed that results derived from sample analysis will not be of direct benefit for the participants. The samples can be linked to the clinical data and therefore also to the name of each individual from whom the sample was collected. However, this study is made under professional secrecy and no personal data will be used during calculations and analysis.

**Experimental design**

*A. actinomycetemcomitans* (JP2 and non-JP2 genotype), *P. gingivalis* and total amount of bacteria in the samples were quantified by qPCR using a Corbett research Rotor Gene 6000 Rotary Analyze machine (Qiagen, Valencia, CA, USA). Specific primers, probes and qPCR programs used, were as described (Kirakodu *et al*., 2008; Yoshida *et
DNA from the samples were previously prepared and stored in a freezer (Höglund Åberg et al., 2012). The qPCR mixtures (10 µL) for quantification of *P. gingivalis* and total amount of bacteria contained 5 µL KAPA SYBER GREEN (KK 4601) (KAPA BIOSYSTEMS, Boston USA), 2 µL template, 2 µL sigma water and 0.5 µmol/L each of *P. gingivalis*-specific primers or universal primers. For quantification of the JP2 and the non-JP2 genotype of *A. actinomycetemcomitans* the corresponding mixture contained 5 µL KAPA SYBER GREEN (KK 4702) (KAPA BIOSYSTEMS, Boston, USA), 2 µL template, 1 µL sigma water and 0.5 µmol/L each of JP2 or non-JP2 specific primers. The JP2 and non-JP2 specific mixture also contained 0.2 µmol/L of the TAMRA- and MGB-probes, respectively (Appendix). Each run included three negative samples (H₂O) and standard mixtures with a given concentration of 10 - 10⁸ bacteria/ml of the studied bacterial species. The Corbette runs resulted in numerous data used for statistical calculations.

**Statistical analysis**

Data analyses and calculations were made in Microsoft Excel 2013 and IBM SPSS Statistics 23 © 2015. The relative risk (RR) was calculated to evaluate the impact of bacterial amount. Since the calculated data was qualitative, Chi-Square tests were used to calculate comparisons between groups with different amounts of *A. actinomycetemcomitans* and development of attachment loss. Because of a skew distribution of data the Spearman correlation test was chosen to calculate any possible correlation between the amounts of *P. gingivalis* and *A. actinomycetemcomitans*. A p-value of < 0.05 was considered statistically significant.

**RESULTS**

**Presence and proportions of *A. actinomycetemcomitans* and *P. gingivalis***

The total bacterial amount in the samples varied greatly with mean proportions of *P. gingivalis* (*n* = 73) and *A. actinomycetemcomitans* (*n* = 128) being 3.7 % (± 11.6) and 6.0 % (± 16.7) respectively.
When looking at the presence, *A. actinomycetemcomitans* was detected in considerably more samples and in higher levels than *P. gingivalis*. In the majority of *P. gingivalis* positive samples *A. actinomycetemcomitans* was detected as well. Among the individuals developing attachment loss 80.0 % were *A. actinomycetemcomitans* positive. However, 78.6 % of those who did not develop attachment loss were *A. actinomycetemcomitans* positive as well. Slight higher proportions of both non-JP2 and JP2 were detected within the group developing attachment loss. Furthermore, the JP2 clone rarely occurred in absence of non-JP2 (Table 1). Regarding the JP2 clone, the majority of the positive samples contained much less than 1 % of the genotype (data not shown).

**Risk for attachment loss development**

Out of 162 analysed samples, 45 belonged to individuals developing attachment loss (≥ 1 site with AL ≥ 3 mm) during the 2-year follow-up period. The relative risk (RR) for development of attachment loss was somewhat lower in the *A. actinomycetemcomitans* low level (< 1 %) group compared to the *A. actinomycetemcomitans* negative group (RR = 0.75; CI [0.36 - 1.55]). A comparison between the negative group and *A. actinomycetemcomitans* medium level (1 - 10 %) group resulted in a slightly elevated risk for attachment loss development in the latter (RR = 1.77; CI [0.91 - 3.46]), yet no significant difference was found (p = 0.085). On the other hand, high levels (> 10 %) of *A. actinomycetemcomitans* was associated with a negligible risk compared to the negative group (RR = 1.06; CI [0.46 - 2.46]). *A. actinomycetemcomitans* in medium levels showed a significant higher risk for attachment loss compared to the low level group (RR = 2.38; CI [1.31 - 4.32], p = 0.005) (Figure 2A).

In comparison with levels < 1 % (*A. actinomycetemcomitans* negative samples included), higher levels (> 1 %) were associated to a significant higher risk (RR = 1.76; [CI: 1.08 - 2.87], p = 0.024) (Figure 2B). Among the individuals developing attachment loss, the JP2 clone occurred in only one individual in proportions exceeding 1 %, hence further calculations based on data from those samples were considered not to be of statistical value (Figure 2C). The JP2 clone was rather commonly found in levels below 1 % in both healthy and diseased individuals at follow up (data not shown).
Correlation
No significant quantitative correlation was found between *A. actinomycetemcomitans* and *P. gingivalis* using Spearman’s rho (p = 0.144). However, high levels of *A. actinomycetemcomitans* appear to correlate to low levels of *P. gingivalis* and vice versa. A considerable part of the samples contained only small amounts of both species and among these low-level samples no distinct correlation could be seen (Figure 3).

Comparison between qPCR and conventional PCR
In the present study, qPCR-technique was used to perform quantitative analyses of the 162 samples with respect to *A. actinomycetemcomitans* and *P. gingivalis*. Among the four JP2 positive samples earlier detected by conventional PCR, two was detected using qPCR. Besides the previous findings, the qPCR-method managed to detect additionally 41 JP2 positive samples. Using qPCR the non-JP2 clone was detected in 85 samples, of which 15 were detected by conventional PCR-technique. Many of the samples positive for *A. actinomycetemcomitans* contained only small bacterial amounts (< 1 %) (data not shown).

DISCUSSION
This study aimed to make quantitative analyses of *A. actinomycetemcomitans* and *P. gingivalis* in samples available from a previous study (Höglund Åberg et al., 2012). The intentions were to identify possible correlations between the two species as well as to study the impact of the amount of *A. actinomycetemcomitans* for development of attachment loss.

In the samples, the total bacterial amount presented large variations, regarding both *P. gingivalis* and *A. actinomycetemcomitans*. Overall, the proportions of *A. actinomycetemcomitans* were higher than the proportions of *P. gingivalis*. The standard deviations were, in both cases, greater than the mean values of bacterial proportions. The large variations in distribution could partly depend on differences in the stage of disease progression among the participants, although considered healthy, at base line. Regardless of attachment loss at follow up, *A. actinomycetemcomitans* was present in the majority of the samples. Even though the detection frequencies of the JP2 clone of *A. actinomycetemcomitans* were slightly higher in the group developing disease, the
increased risk, if any, was not substantial. This contradicts earlier studies describing a strong association between presence of this species and increased risk for development of attachment loss (Höglund Åberg et al., 2014).

In the majority of JP2 positive samples, much less than 1 % of the genotype was found both in the healthy and the diseased group. The presence of JP2 did not indicate a markedly increased risk for development of attachment loss. This do not coincide with previous findings where the presence of JP2, in not known proportions, have been associated with development of periodontitis (Haubek et al., 2004; 2008; Höglund Åberg et al., 2014; Rylev et al., 2011). However, since this study is of minor extent compared to other studies in the same area, these results have limited value.

When looking at the proportions of bacteria as a factor for disease development, a significant difference in progress of attachment loss was found between the low (< 1 %) and medium (1 - 10 %) groups containing A. actinomycetemcomitans (p = 0.005). This indicates that presence of A. actinomycetemcomitans in medium levels constitutes the highest risk for development of periodontal disease. Between the proportions < 1 % and > 1 % of the bacteria a significant difference was found as well (p = 0.024). It is somewhat surprising that the group with the highest levels (> 10 %) of A. actinomycetemcomitans did not exhibit higher disease incidence compared to the negative group. The implications from this could be that presence of A. actinomycetemcomitans in certain levels is of importance for attachment loss development. In a recent study it is suggested that A. actinomycetemcomitans levels increase in the initiating stage of disease, before attachment loss can be clinically measured. As the pocket deepens A. actinomycetemcomitans is replaced by obligate anaerobe species (Höglund Åberg et al., 2015). The results from this study shows that medium proportions of A. actinomycetemcomitans detected in healthy adolescents contributes the highest risk for developing attachment loss. High levels, however, do not contribute to further increased risk. Further sectioning of the medium level group could give additional knowledge regarding critical bacterial levels for development of attachment loss and periodontal disease.
A previous study has shown that *P. gingivalis* has characteristics which can inhibit and outcompete *A. actinomycetemcomitans* (Johansson et al., 2000). Other studies have suggested that *A. actinomycetemcomitans* has a considerable role at initiating periodontal disease and that *P. gingivalis* replaces *A. actinomycetemcomitans* as the periodontal pocket gets deeper (Dahlén et al., 2014; Höglund Åberg et al., 2015). The data mentioned above indicates a possible negative correlation between the two species. The analyses in the present study did not, to some surprise, show any significant correlation (p = 0.144) concerning the amounts of *A. actinomycetemcomitans* and *P. gingivalis*. However, the results do indicate a slight negative correlation between high levels of *A. actinomycetemcomitans* and *P. gingivalis* in the same sample. Also, it is notable that qPCR is a method which detects DNA from both living and dead bacteria which could affect the result. Further studies based on culturing would be of interest to make specific analysis of the correlation between living bacteria only. Since the study population included in the present study was narrow, the importance of the results is of limited value. Out of 315 available samples 153 has not yet been successfully analysed, due to the time frame of this study. Further analyses of the 153 remaining samples may affect the signification of the result. Thus, the lack of a significant negative correlation in this study, does not rule out the possibility that such relation between *A. actinomycetemcomitans* and *P. gingivalis* do exist.

The periodontal samples used in this study have previously been analysed for the presence of both non-JP2 and the JP2 clone of *A. actinomycetemcomitans* using culturing and conventional PCR (Höglund Åberg et al., 2012; 2014). The samples have not, however, been quantitatively analysed with qPCR. The results from this study indicate that qPCR is a highly sensitive method, compared to conventional PCR, for detection both of the non-JP2 and JP2 clone of *A. actinomycetemcomitans*. Many of the *A. actinomycetemcomitans* positive samples contained small amounts (< 1 %), which may have contributed to the lower detection level with conventional PCR. It is somewhat questionable though, whether the low detection levels provided by qPCR contribute to any clinical information of importance.
Concluding remarks
The results from this study suggest that bacterial amounts of *A. actinomycetemcomitans* exceeding 1 % are related to attachment loss development. Medium levels (1 - 10 %) are particularly associated with increased risk for periodontal disease initiation and progression. This study failed to find a significant negative correlation between *A. actinomycetemcomitans* and *P. gingivalis*. To provide more knowledge about the relation between *A. actinomycetemcomitans* and *P. gingivalis in vivo*, further studies are required. It would be of interest to analyse the remaining samples from the initially healthy individuals as well as the individuals exhibiting attachment loss at base line.

ACKNOWLEDGEMENTS
We would like to share our gratitude with our tutors Anders Johansson, Rolf Claesson and Carola Höglund Åberg for excellent supervising and encouragement during the entire process. We also thank Christine Roth for providing great laboratory assistance.
REFERENCES


TABLES

**Table 1.** Detection frequencies of *A. actinomycetemcomitans* (Aa) and *P. gingivalis* (Pg) in 162 samples from Ghanaian adolescents using qPCR

<table>
<thead>
<tr>
<th></th>
<th>Pg</th>
<th>Aa</th>
<th>Pg and Aa</th>
<th>Non-JP2</th>
<th>Only non-JP2</th>
<th>JP2</th>
<th>Only JP2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
</tr>
<tr>
<td><strong>All samples</strong></td>
<td>162</td>
<td>73 (45.1)</td>
<td>128 (79.0)</td>
<td>62 (38.3)</td>
<td>121 (74.7)</td>
<td>83 (51.2)</td>
<td>45 (27.8)</td>
</tr>
<tr>
<td><strong>CAL &lt;3mm</strong></td>
<td>117</td>
<td>48 (41.0)</td>
<td>92 (78.6)</td>
<td>42 (35.9)</td>
<td>87 (74.3)</td>
<td>64 (54.7)</td>
<td>28 (23.9)</td>
</tr>
<tr>
<td><strong>CAL ≥3mm</strong></td>
<td>45</td>
<td>25 (55.6)</td>
<td>36 (80.0)</td>
<td>20 (44.4)</td>
<td>34 (75.6)</td>
<td>19 (42.2)</td>
<td>17 (37.8)</td>
</tr>
</tbody>
</table>

*CAL ≥ 3 mm signifies disease progression.*
**Figures**

**Figure 1.** Flow chart of study population and samples. At base line 500 adolescents were examined clinically and microbial samples were collected. The present study included individuals lacking attachment loss at base line and available at follow up (n = 315). 162 of the analysed samples fulfilled the criteria for inclusion.
Figures 2. Proportions of *A. actinomycetemcomitans* related to development of attachment loss (CAL ≥ 3 mm) at the 2-year follow-up examination. (A) Bacterial proportions grouped as negative (0 %, n = 9), low (< 1 %, n = 14), medium (1 - 10 %, n = 15) and high (> 10 %, n = 7). Medium levels of *A. actinomycetemcomitans* showed the strongest association to attachment loss development. (B) Levels exceeding 1 % (n = 22) were associated to a significantly (p = 0.024) higher risk for attachment loss development than levels below 1 % (n = 23). (C) Unlike the non-JP2 group (n = 21), JP2 in levels ≥ 1 % (n = 1) did not indicate an increased risk of developing attachment loss compared to generally low levels (< 1 %) of *A. actinomycetemcomitans* (n = 14) and *A. actinomycetemcomitans* negative samples (n = 9).
Figure 3. Correlation between *A. actinomycetemcomitans* and *P. gingivalis* in 162 samples from adolescents in Ghana. Graphically high levels of *P. gingivalis* corresponded to low levels of *A. actinomycetemcomitans* and conversely which indicates a slight negative correlation between the two species. Concerning low levels the correlation was not as evident. No significant correlation between the two species was found (p = 0.144).
### APPENDIX

Primer-pairs for quantification of total amount of bacteria and *P. gingivalis* (Kirakodu *et al.*, 2008)

<table>
<thead>
<tr>
<th></th>
<th>Universal</th>
<th><em>P. gingivalis</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Forward</strong></td>
<td>GATTAGATACCTGCTAGTCCAC (116)</td>
<td>AGGCAGCCTGCCCTACTGCG (108)</td>
</tr>
<tr>
<td><strong>Reverse</strong></td>
<td>TACCTGTAGACCTT (117)</td>
<td>ACTGTAGCAACTCGATGT (109)</td>
</tr>
</tbody>
</table>

Primer-pairs and probes for quantification of *A. actinomycetemcomitans* non-JP2 clone and JP2 clone (Yoshida *et al.*, 2012)

<table>
<thead>
<tr>
<th></th>
<th><em>A. actinomycetemcomitans, non-JP2</em></th>
<th><em>A. actinomycetemcomitans, JP2</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Forward</strong></td>
<td>CGCAAGTGCCATAGTATCCACT (Non-JP2F; 142)</td>
<td>TCTATGAAACTGAAAACCTCGAGAAT (JP2-F3; 140)</td>
</tr>
<tr>
<td><strong>Reverse</strong></td>
<td>TCGTCTGCTACTATAAGCAGAGAG (Non-JP2R; 143)</td>
<td>GAATAAGATAACCCACAAATCCATCC (JP2-R2; 141)</td>
</tr>
<tr>
<td><strong>Probe</strong></td>
<td>FAM-ATATTGTGTACATCGCCC-MGB</td>
<td>FAM-AACAAATCGTGGCATTTCGCGGAA-TAMRA</td>
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</tbody>
</table>

Cycle settings for quantification using Corbette research Rotor-Gene 6000 Rotary Analyse (QIAGEN, Valencia, CA, USA)

<table>
<thead>
<tr>
<th>Primer/Probe</th>
<th>Universal</th>
<th><em>P. gingivalis</em></th>
<th>Non-JP2, JP2</th>
</tr>
</thead>
<tbody>
<tr>
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<td>95°/600 sec</td>
<td>95°/600 sec</td>
</tr>
<tr>
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<td>95°/10 sec</td>
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<td>60°/5 sec</td>
<td>58°/40 sec</td>
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<tr>
<td><strong>Cycling/time</strong></td>
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<td>72°/16 sec</td>
<td>72°/1 sec</td>
</tr>
</tbody>
</table>