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Development and evaluation of a saliva-based chair-side diagnostic for the detection of *Porphyromonas gingivalis*

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Porphyromonas gingivalis is a key pathogen in the polymicrobial biofilm that is associated with the oral disease chronic periodontitis. A number of studies have shown that in humans the level of *P. gingivalis* in the polymicrobial biofilm is positively correlated with disease progression. The aim of this study was to develop a *P. gingivalis* diagnostic that has high specificity and sensitivity for *P. gingivalis* using a range of laboratory and clinical isolates and then compare the efficacy of the diagnostic with RTPCR using samples from chronic periodontitis patients and age- and sex-matched healthy controls. Key parameters for the kit were to use saliva as the biological fluid as this is a most convenient medium for chair-side sampling and to give a positive reading for the reported threshold for detection of 5×10^5 *P. gingivalis* cells/mL that indicates disease progression. We initially screened a range of monoclonal antibodies for recognition of the *P. gingivalis* conserved virulence factor RgpA-Kgp complex and identified two mAbs that could be used in a capture and detection ELISA system. These mAbs were used to formulate and manufacture the GC *P. gingivalis* saliva diagnostic kit used in the study. To validate the saliva kit, saliva (*P. gingivalis* free) was spiked with known concentrations of viable *P. gingivalis* whole cells of W50, 381, A7A1-28, and ATCC 33277; *P. gingivalis* clinical isolates; *P. gingivalis* vesicles; and the secreted form of the RgpA-Kgp complex. Laboratory findings indicated that the kit was able to detect all laboratory and clinical isolate strains of *P. gingivalis* at 5×10^4 /mL to 5×10^5 /mL. It was also able to detect the RgpA-Kgp complex and vesicles at 5×10^4 and 5×10^5 cell equivalent doses, respectively. Saliva and plaque were then collected from 50 subjects with moderate–severe chronic periodontitis and 50 age- and sex-matched subjects with healthy periodontium. Real-time PCR was utilised to analyse levels of *P. gingivalis* in both saliva and plaque. The saliva kit was found to give a positive result within 90 seconds. Using point bi-serial correlation analysis, a significant ($p = 0.04$) correlation was found for detection of *P. gingivalis* using the saliva kit and *P. gingivalis* levels in saliva and plaque as determined by real-time PCR. A sensitivity of 92% and a specificity of 96% were found when compared to real-time PCR at a 10^5 *P. gingivalis* cell threshold.

In conclusion, the *P. gingivalis* saliva kit was shown to be rapid and has a comparable detection capacity to real-time PCR. Thus, the *P. gingivalis* saliva diagnostic has the potential to be a simple and time-efficient chair-side diagnostic for the detection of *P. gingivalis*.