invivo®

Oral EcologiX™

Oral Health & Microbiome Profile

Phylo Bioscience Laboratory

—

INTERPRETIVE GUIDE

Oral EcologiX™

INTRODUCTION

Due to recent advancements in culture-independent techniques, it is now possible to measure the composition of the human microbiota. The oral cavity is a complex ecosystem, comprising several habitats including the teeth, gums, tongue and tonsils, all colonised by bacteria[1]. The oral microbiota is comprised of approximately 600 taxa at the species level, with different groups and subsets inhabiting different niches.

The microbiota of the oral cavity exists as a complex biofilm that remains stable despite environmental changes. However, dysbiosis, in form of infection, injury, dietary changes and risk-associated factors (e.g. smoking) may disrupt the biofilm community, favouring colonisation and invasion of pathogens. Disruption of the biofilm community to a pathogenic profile, induces host immune responses, chronic inflammation and ultimately development of local and systemic diseases. However, much of this damage is reversible if pathogenic communities are removed, and homeostasis is restored.

To this end, Phylobioscience have developed the Oral EcologiX™ oral health and microbiome profile, a ground breaking tool for analysis of oral microbiota composition and host immune responses. Using innovative microbial culture-independent technologies, including quantitative real-time PCR (qRT-PCR) and enzyme-linked immunosorbent assay (ELISA), the profile provides an accurate, reliable and quantifiable measurement of microbiota abundance and host inflammatory markers.

For microbiota composition analysis, the technology detects:

- Abundance of commensal bacteria
- Abundance of caries-associated bacteria
- Abundance of periodontitis-associated bacteria
- Presence of viral pathogen herpes simplex virus 1 and 2 (HSV-1)
- Presence of human papillomavirus-16 genotype (HPV-16)

For host biomarker analysis, the technology detects:

- IL-1β (marker for inflammation)

Dependent on microbiota composition and host biomarkers, the Oral EcologiX™ profile will report three different states:

- Healthy oral microbiome (homeostasis of host and microbiome)
- Oral dysbiosis detected (imbalance of the oral microbiota and/or host immune response)
- Oral pathogen detected (HSV-1, HPV-16)

GLOSSARY

Term	Description	References
Commensal	Microorganism (i.e. bacteria, fungi) that lives in symbiosis with the host when residing within its specific environment	1
Pathogen	Microorganism (e.g. bacteria, fungi, virus) that may cause disease	1
Homeostasis	Ability to maintain internal stability in an organism despite environmental changes	2
Dysbiosis	Imbalance or disturbance in the human microbiota	3
Microbiota	Collective ecosystem of microorganisms that inhabit the human body	4
Periodontitis	Periodontal disease (PD), or periodontitis, is a chronic inflammatory disease of the periodontium - the tissues that surround and support the teeth	5
Caries	Demineralisation and destruction of enamel and tooth structure by acid-producing caries-associated bacteria (i.e. *Streptococcus mutans*)	2
Gingivitis	Inflammation, irritation and swelling of the gingiva - the part of the gum that surrounds the base of teeth	6
Orange complex	Microbial community associated with periodontitis. Bacteria in the orange complex are recognised as 'bridge species" that bridge non-pathogenic bacteria to pathogenic species through cell-cell interactions	7
Red complex	Microbial community associated with periodontitis. Red complex bacteria include *Porphyromonas gingivalis, Treponema denticola*, and *Tannerella forsynthia* and are recognised as the most important pathogens in periodontal disease	7

BACKGROUND

Oral Microbiota

The human oral microbiome database (HOMD) is the first curated description of human associated microbiome and provides comprehensive information on the approximately 1,000 species that inhabit the human oral cavity (HOMD). The oral microbiota is a complex ecosystem comprised of bacteria, fungi, archaea, viruses, and protozoa, with bacteria being the most studied[8,9]. Bacteria are found on all oral tissues and the composition varies significantly with age[10]. In a recent study, 16S rRNA analysis detected 619 phyla in the oral microbiota; the six main phyla were *Firmicutes, Bacteroidetes, Proteobacteria, Actinobacteria, Spirochaetes,* and *Fusobacteria*, which comprised 96% of the taxa. The remaining phyla, *Euryarchaeota, Chlamydia, Chloroflexi,* SR1, *Synergistetes*, *Tenericutes*, and TM7, comprised the remaining 4% of the taxa[8]. Bacteria associated with periodontal health include *Streptococcus, Granulicatella, Neisseria, Haemophilus, Corynebacterium, Rothia, Actinomyces, Prevotella,* and *Capnocytophaga*[11].

Homeostasis

The majority of oral microbiota species exist within a complex multispecies structure termed the oral biofilm (dental plaque). The development of oral biofilms is initiated by adherence of pioneer species to salivary proteins and glycoproteins adsorbed onto tooth enamel. In healthy individuals, the oral biofilm is dominated by commensal bacteria that maintain homeostasis through intricate interactions with host immunity and other microorganisms[12]. However, when equilibrium is compromised (drugs, diet, injury, infection, etc.) and microbial imbalance occurs, pathogenic bacteria may colonise and cause pathologies such as dental caries or periodontitis[13].

Early colonisers of the mouth are commensal *Streptococci* spp. including *S. mitis*, *S. sanguinis* and *S. gordonii*. These early colonisers are able to bind to the tooth surface and prevent colonisation of other bacteria through production of bacteriocins, H_2O_2 and alkali production[2]. In the absence of a carbohydrate-rich diet, commensal *Streptococci* remain at high levels within dental plaque and maintain homeostasis. Dominance of commensal *Streptococci* is associated with good oral health[2].

Late colonisers, including Gram-negative (i.e. *Veillonella* spp.) and Gram-positive species (i.e. *Actinomyces* spp.) build up on the initial Streptococcal biofilm via cell-cell interactions. This series of co-aggregation and co-adhesion events leads to the formation of a mature biofilm, a relatively stable community where all species exist in homeostasis[14]. The disruption of homeostasis, termed dysbiosis, has been linked to a multitude of conditions including dental caries, gingivitis, periodontitis and increased risk of oral cancer[15].

Dysbiosis

Periodontal Disease

Periodontal disease (PD), or periodontitis, is a chronic inflammatory disease of the periodontium, the tissues that surround and support the teeth[5]. It affects 10–15% of adults and is the most common cause of tooth loss worldwide. Periodontal disease has a polymicrobial aetiology within the framework of a complex microbial ecosystem. It is caused by a synergistic and dysbiotic biofilm community, with keystone pathogens such as *Porphyromonas gingivalis* initiating the disruption of tissue homeostasis[16]. The consortium of bacteria most strongly implicated in the pathogenesis of PD is *P. gingivalis, T. denticola* and *T. forsythia* (red complex), all three of which, are routinely found in subgingival plaque in patients with chronic periodontitis[7] (Figure 1).

PD is characterised by the destruction of the periodontal ligament, connective tissue and alveolar bone as a result of chronic immune and inflammatory responses. Inflammation in periodontitis is predominantly mediated by IL-1, IL-8, TNF-α, prostaglandins and matrix metalloproteinases (MMPs)[17]. These mediators are proposed to affect functions and activities of leukocytes, osteoblasts and osteoclasts, promoting tissue remodelling locally and systemically[18,19]. Susceptibility to periodontal disease is influenced by host genotype, stress, diet and associated behaviour, including smoking[20].

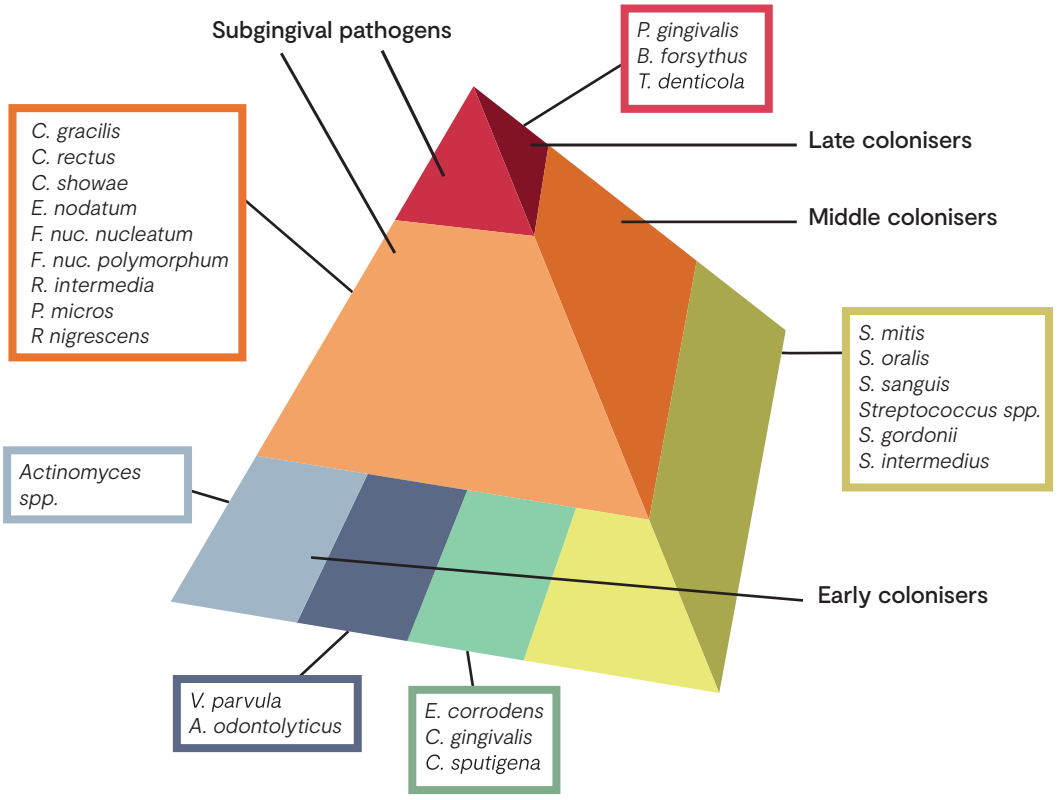


Figure 1: Periodontitis-associated bacteria grow as synergistic and multispecies communities comprised of different functional complexes dependent on pathogenicity. Figure adapted from Haffajee and Socransky (1994).

Early colonisers include members of the yellow, green, and purple complexes (oral health). The orange complex bacteria generally appear after the early colonisers and include many putative periodontal pathogens, such as *Fusobacterium nucleatum*. The orange complex includes bacteria which, as "bridge species", form a link between the early colonisers and the highly pathogenic bacteria of the red complex. The pathogenic potential of orange complex bacteria is significantly increased due to the production of various toxins and enzymes. Occurrence of bacteria of the red complex is characteristic of the final colonisation phase, which culminates in the development of a structured, stable bacterial community (climax community) and includes putative periodontal pathogens. Colonisation with these bacteria, which are significantly involved in the destruction of the periodontium, builds on the presence of the less pathogenic species mentioned above. Their ability to penetrate tissue also makes treatment difficult[21].

Caries

Caries is the most common chronic childhood infection and can lead to progressive destruction of dental hard tissue. Risk factors for caries include the frequent consumption of fermentable dietary carbohydrates (especially sucrose) and/or reduced saliva flow. The increased intake of carbohydrates, alongside poor oral hygiene, leads to increased production of extracellular glucan matrix by oral *Streptococci*[2]. This thickening matrix encapsulates bacteria and creates acidic microenvironments via increased carbohydrate fermentation, that are not readily permeated by saliva. This increasingly acidic environment is not tolerated by commensal *Streptococci* spp. and favours growth of acid-tolerant, caries-associated species including *Streptococcus mutans*, *Veillonella* spp. and *Lactobacillus* species. Species of *Lactobacillus* associated with childhood dental caries include *L. rhamnosus, L. casei,* and *L. pseudoplantarum*[22]. Species associated with adulthood caries include *L. casei, L. fermentum* and *L. rhamnose* [23,24]. If excessive carbohydrate consumption and poor oral hygiene is prolonged, this results in development of a thick glucan matrix, extremely acidic microenvironments and unrepairable damage to tooth enamel and the onset of clinical disease[2].

Oral Cancer

Oral cancer is one of the ten most prevalent cancers in the world, with more than 90% of mouth neoplasms being oral squamous cell carcinoma (OSCC), originating from the oral mucosa. The pathogenesis of OSCC is attributed mainly to smoking, high alcohol intake and smokeless edible tobacco products. However, other risk factors include viral infections, infection with *Candida* species, periodontitis, poor oral hygiene and chronic bacterial infections and inflammation[25]. Human papillomavirus virus plays a significant role in the development of oral cancer. HPV-16 genotype is the most common genotype to persist in the oral mucosa and is the most common genotype isolated from oral squamous cell carcinomas[26].

There is an increasing body of evidence supporting a role for *S. intermedius* in oral cancer. In a recent study using biopsies of patients with oral squamous cell carcinoma, *S. intermedius* was detected in 70% of both non-tumour and tumour sites[25]. *T. denticola* has also been detected in oral and gastrointestinal tumour samples[27].

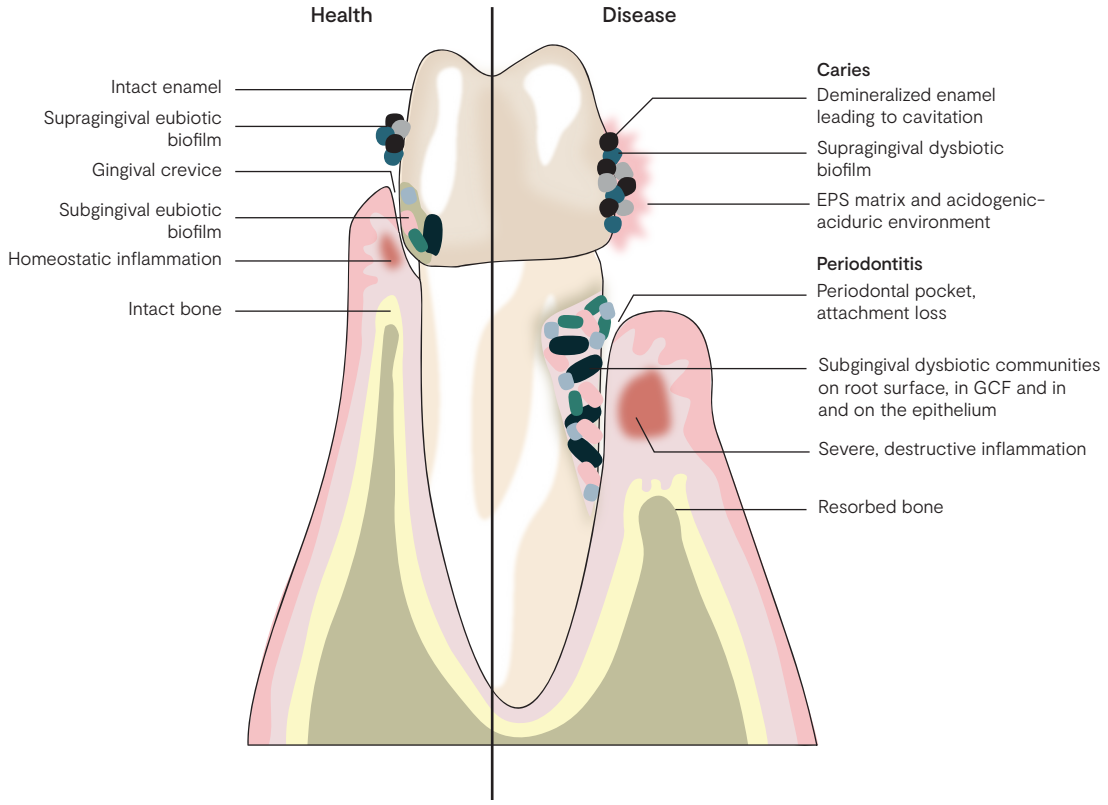


Figure 2: Development of disease in the oral cavity. Oral health involves colonisation of the enamel and gingival crevice by commensal *Streptococci* and bacteria. Dysbiosis involving increased intake of carbohydrates, infection or injury facilitate

colonisation of caries-associated and acid-producing bacteria including *Streptococcus mutans,* causing destruction of the enamel. Dysbiosis may also lead to microbial invasion of the mucosa and further development of subgingival biofilm, involving colonisation by "red complex" pathogens including *P. gingivalis* and *T. denticola,* leading to inflammation and destruction of tooth supporting tissues. Figure adapted from Lamont *et al.* (2018)[28].

SYSTEMIC DISORDERS

There is a growing body of research to support a role for PD pathogens as contributing factors for numerous systemic diseases, including cardiovascular disease (CVD), diabetes, adverse pregnancy outcomes, rheumatoid arthritis (RA) and Alzheimer's disease (AD)[29–33]. The proposed aetiology involves direct and indirect effects of oral bacteria on host immune responses, inflammation and therefore development of systemic disorders.

1. Direct mechanism: In chronic periodontitis, periodontal pockets become ulcerated providing direct entry points for oral bacteria into the systemic circulation. Circulating bacteria (i.e. *P. gingivalis; T. forsynthia*) may then induce direct effects on organs and tissues.

2. Indirect mechanism: Chronic inflammation has been implicated in a multitude of disorders including diabetes, obesity and autoimmune disorders. Chronic inflammation associated with chronic periodontitis may represent a source of inflammation, able to induce and acerbate systemic disorders.

Pregnancy

F. nucleatum is the most prevalent oral species implicated in adverse pregnancy outcomes (i.e. miscarriage, preterm labour)[34]. It has been detected in a wide variety of placental and foetal tissues including the amniotic fluid and foetal membranes[35]. It is proposed that *F. nucleatum* translocates from the maternal oral cavity to the intrauterine cavity via hematogenous transmission[36]. *C. rectus* is also associated with adverse pregnancy outcomes, including preterm delivery[37].

Cardiovascular

S. mutans is associated with bacteraemia and infective endocarditis (IE)[38]. These oral bacteria can disseminate into the bloodstream via professional dental treatment and tooth brushing. Indeed, in a review of 848 cases of iE, *Streptococcus* spp. were the most common microorganisms isolated (49.5%)[39], and *S. mutans* is recovered from 15% of patients with streptococcal valvular disease[40]. The ability of *S. mutans* to produce amyloid as part of the oral biofilm has also implicated this bacterium in dementia[41]. Furthermore, collagen binding surface Cnm protein, expressed on cnm-positive *S. mutans*, has been associated with cerebral microbleeds (CMBs) - an important risk factor for strokes and dementia. In a screen of 279 community residents, cnm-positive *S. mutans* was detected more frequently in patients with CMBs and the risk of CMBs was higher in the group with cmb-positive *S. mutans* [42]. Additionally, systemic infection by *Actinobacillus actinomycetemcomitans* has been associated with atherosclerosis[43], brain abscesses[53], and endocarditis[44].

Neurodegeneration

Periodontal pathogens, *P. gingivalis* and *T. denticola* are also associated with the development of Alzheimer's disease (AD)[45]. LPS of *P. gingivalis* was detected in the brain tissue of AD patients[46]. In addition, in a recent study using a mouse model for periodontitis, after infection a significant number of *P. gingivalis* genomic DNA was detected in the brain[47].

Arthritis

P. gingivalis has also been implicated in the pathogenesis of rheumatoid arthritis (RA). *P. gingivalis* expresses peptidyl arginine deiminase (PAD), which mediates citrulation of peptides. Systemic circulation of citrulated peptides is a key risk factor for development of RA-associated inflammation[95]. Furthermore, significantly higher anti-*P. gingivalis* antibodies have been detected in patients with RA, compared to systemically and periodontally healthy controls[48].

IBD

Fusobacterium nucleatum has been linked to colorectal cancer (CRC). It is found in high amounts in CRC carcinomas, adenomas, in stools of patients with carcinoma and in rectal swabs of CRC patients[49,50]. This bacterium has also been detected in colonic biopsies of IBD patients [51], and strains isolated from inflamed tissues of IBD patients are more virulent than those from healthy individuals[52].

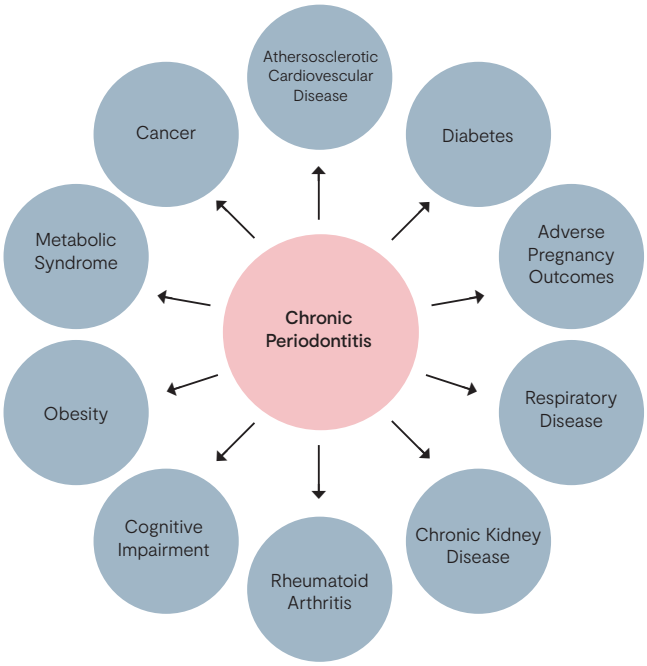


Figure 3: Associations between periodontitis and systemic disease. Figure adapted from Winning and Linden (2015)[53].

METHODOLOGY

qRT-PCR FOR QUANTIFICATION OF ORAL MICROBIOTA SPECIES

The Phylobioscience Oral EcologiX™ profile utilises real-time quantitative PCR (qRT-PCR) for analysis of oral microbiota populations. qRT-PCR is used to quantify the number of copies of a gene of interest in a community sample. Real time qPCR reactions are performed using Taqman technology.

ELISA ASSAY FOR MEASUREMENT OF PRO-INFLAMMATORY MARKERS

BIOMARKER	TYPE	CLASSIFICATION
HOST IMMUNE MARKERS		
IL-1β	Pro-inflammatory cytokine	N/A
MICROBIOTA		
Commensal fungi		
Candida albicans	Fungi	N/A

Bacteria

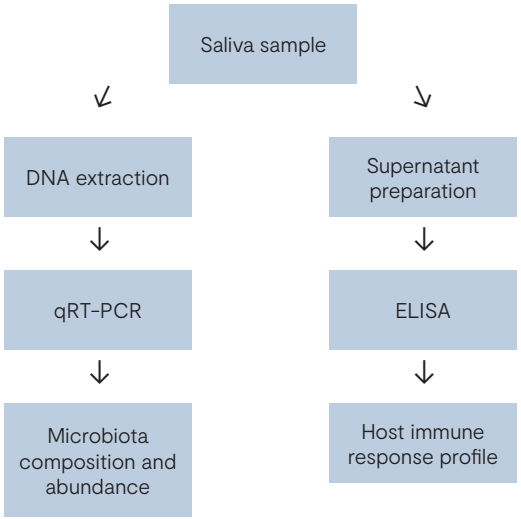
Propionibacterium acidifaciens	Bacteria	Gram-positive
Lactobacillus spp.	Bacteria	Gram-positive
Streptococcus mutans	Bacteria	Gram-positive
Eubacterium nodatum	Bacteria	Gram-positive
Peptostreptococcus anaerobius	Bacteria	Gram-positive
Parvimonas micra	Bacteria	Gram-positive
Porphyromonas gingivalis	Bacteria	Gram-positive
Fusobacterium nucleatum	Bacteria	Gram-negative
Aggregatibacter actinomycetemcomitans	Bacteria	Gram-negative
Treponema denticola	Bacteria	Gram-negative
Actinobacillus actinomycetemcomitans	Bacteria	Gram-negative
Campylobacter rectus	Bacteria	Gram-negative
Tannerella forsythia	Bacteria	Gram-negative
Prevotella intermedia	Bacteria	Gram-negative
Prevotella nigrescens	Bacteria	Gram-negative

Viral pathogens

HSV-1	Virus	N/A
HSV-2	Virus	N/A
HPV-16_E6	Virus	N/A
HPV-16_E7	Virus	N/A

Table 1: Oral EcologiX™ biomarkers

METHODOLOGY FLOWCHART



INTERPRETATION OF DATA

Host Biomarkers

IL-1β	HEALTHY: <300pg/ml	HIGH: >300pg/ml

Interleukin 1-β (IL-1β) is a key mediator of the inflammatory response and a master cytokine that regulates induction of other cytokines, including IL-8[54]. Produced by innate immune cells, it is crucial for host responses against infection and injury. IL-1β is synthesised in response to inflammatory stimuli from pathogens, stress conditions, and other danger signals. IL-1β is a major mediator of inflammation in periodontitis[55].

Microbiota Profiles

Fungi

Candida albicans	COMMENSAL FUNGI	Overgrowth associated with oral candidiasis

Candida albicans is a fungal species of the commensal oral microbiota and occurs in the oral cavity of 30-100% of healthy individuals[9,63]. The relative abundance of *Candida* species in the oral cavity is influenced by geographic location, health status and diagnosis and exposure to antifungal drugs[64,65]. Additionally, the rate of carriage increases with age; *C. albicans* are recovered from 60% of dental patient's mouths over the age of 60 years[66].

C. albicans is an opportunistic pathogen and can cause oral candidiasis in immunocompromised hosts. Oral candidiasis is associated with changes in oral fungal biodiversity, increased nutrient availability, overgrowth of *Candida* species and transition from yeast to hyphal growth mode, termed phenotypic switching[67]. Several studies have reported increased prevalence of subgingival *C. albicans* in chronic periodontitis patients compared to healthy individuals[68,69].

Gram-positive bacteria

Lactobacillus spp.	COMMENSAL BACTERIA	Associated with dental caries

Lactobacillus spp. are Gram-positive, rod shaped bacteria that comprise a normal member of the human microbiota, inhabiting multiple environments including the gut, genital tract and oral cavity. Lactobacilli were the first microorganisms implicated in dental caries[73], and subsequent studies reported a strong correlation between *Lactobacillus* counts and dental caries[74,75]. Lactobacilli have been reported to occur in high numbers in both superficial and deep caries [76].

Previous studies using 16S rRNA sequencing has reported dominant species in child and adult caries to include *L. fermentum, L. rhamnosus, L. gasseri, L. casei* and *L. salivarus*. Less common species include *L. mucosae, L. crispatus,* and *L. ultunesis*[77-79]. Low levels of Lactobacilli have been reported in dental plaque from caries-free children[80].

Streptococcus mutans	COMMENSAL BACTERIA	Associated with dental caries and systemic diseases

The key habitats for *S. mutans* are the mouth, pharynx and intestine. *S. mutans* has a key role in the aetiology of dental caries as they can adhere to the enamel salivary pellicle and to other plaque bacteria[83]. Appearance of *S. mutans* in the tooth cavity is typically followed by caries after approximately 6-24 months[84]. Acidogenic *S. mutans* is able to form the structural biofilm component, exopolysaccharide (EPS), in the presence of sucrose, fructose and glucose.

In addition to caries, *S. mutans* is linked to a wide range of systemic diseases and disorders including bacteraemia, infective endocarditis and dementia[38-42].

Eubacterium nodatum	PUTATIVE PATHOGEN	Isolated from patients with severe periodontitis

Eubacterium nodatum are Gram-positive, anaerobic bacteria isolated from patients with moderate and severe adult periodontitis. In a recent study characterising microbiota profiles associated with peri-implantitis, *E. nodatum* was shown to be among the most prevalent species detected[85]. A strong association was shown in periodontitis with *E. nodatum* and *T. denticola* in the presence, or absence, of high levels of core periodontitis pathogens[86].

Parvimonas micra	COMMENSAL BACTERIA	Associated with periodontitis and systemic diseases

Originally classified as *Peptostreptococcus micros*, the Gram-positive anaerobic cocci currently classified as *Parvimonas micra,* is a normal member of the gut and oral microbiota. *P. micra* has been implicated in periodontal disease and infections outside the oral cavity[87]. *P. micra* shows significantly higher prevalence in patients with periodontitis[88]. There is a growing body of research that supports the role of *P. micra* as the single causative agent of spondylodiscitis (rare spine infection)[87,89,90]. In rare cases, *P. micra* has been identified as the single causative agent in infectious endocarditis[91].

Peptostreptococcus anaerobius	COMMENSAL BACTERIA	Associated with periodontitis

Gram-positive anaerobic cocci frequently cultured from the mouth, upper respiratory tract, skin and soft tissues, bones and joints, GI tract and genitourinary tracts[92]. *P. anaerobius* is associated with periodontitis and has been detected in periodontal disease-associated biofilm[4]. It has been isolated from patients with gingivitis, chronic periodontitis and aggressive periodontitis[4].

Gram-negative bacteria

Fusobacterium nucleatum	COMMENSAL BACTERIA	Associated with periodontitis and systemic disorders

Fusobacterium nucleatum is a Gram-negative obligate anaerobe bacterium that inhabits the oral cavity. *F. nucleatum* is one of the most abundant species in the oral cavity in both diseased and healthy individuals[58]. It is implicated in different types of periodontal diseases including gingivitis, chronic periodontitis, localised aggressive periodontitis and generalised aggressive periodontitis[58–60]. Prevalence of this bacterium increases with the severity of disease, inflammation and pocket depth[5,61].

F. nucleatum is the most prevalent oral species implicated in adverse pregnancy outcomes (i.e. miscarriage, preterm labour)[34]. *F. nucleatum* has also been linked to colorectal cancer[49,50] and IBD [51,52]. *F. nucleatum* is a potent stimulator of inflammatory cytokines, IL-6, IL-8, and TNF-α[62].

Porphyromonas gingivalis	PATHOGEN	Major periodontitis pathogen

Porphyromonas gingivalis is a Gram-negative anaerobe and is one of the major pathogens associated with chronic periodontitis. Recent work indicates that *P. gingivalis* functions as a keystone organism in periodontitis, i.e. it is an organism able to hold an entire arch/community together. Through expression of a wide range of virulence factors, *P. gingivalis* induces inflammation, evades the host immune response and stimulates bone reabsorption. Local inflammation induces dysbiosis and imbalance of the plaque (biofilm) microbiota[93]. This pathophysiology is recognised as a key initiating event for periodontitis[94].

P. gingivalis, T. forsynthia and *T. denticola* are categorised as the 'red complex' because they are the most frequently isolated species from chronic lesions in chronic periodontitis[7]. There is also a growing body of evidence linking this pathogen to Alzheimer's Disease, and rheumatoid arthritis[47,48,95]. An association between elevated *P. gingivalis* serum IgG antibodies and increased risk of mortality from oriodigestive cancer has also been reported[96].

Aggregatibacter actinomycetemcomitans	COMMENSAL BACTERIA	Associated with periodontitis and systemic diseases

Gram-negative oral pathobiont that causes localised aggressive periodontitis (LAP), characterised by rapid destruction of tooth supporting tissues[97]. LAP has a prevalence of approximately 0.5%, but is 15 times more prevalent in African-American adolescents, compared to the general population[98]. *A. actinomycetemcomitans* is also associated with a range of systemic diseases, including infections of the heart[99], urinary tract[100] and brain[97,101].

Treponema denticola	PATHOGEN	Major periodontitis pathogen

Gram-negative spirochete bacteria frequently isolated from human periodontal lesions. Its role in pathogenesis and progression of periodontitis has been widely reported[102]. *P. gingivalis*, *T. forsynthia* and *T. denticola* are categorised as the 'red complex' because they are the most frequently isolated species from chronic lesions in chronic periodontitis[7]. *T. denticola* possess several virulence factors that are implicated in periodontal pathogenesis[103,104]. *T. denticola* is associated with Alzheimer's disease [45] and oral and gastrointestinal cancer development [27,105].

Actinobacillus actinomycetemcomitans	PATHOGEN	Associated with periodontitis and systemic diseases

A. actinomycetemcomitans is commonly found in the oral microbiota; however, it is recognised as a periodontal pathogen involved in the development of aggressive periodontitis[106,107]. Carriage rates of *A. actinomycetemcomitans* can be up to 20% of the population[108]. Seven serotypes exist, with large variations in their surface antigens and therefore, virulence and pathogenicity[109]. It has been proposed that *A. actinomycetemcomitans* colonises the oral mucosa and translocates to the gingival margin, inducing host immune responses and local inflammation, subsequently leading to degradation of tooth supporting tissues[110]. Systemic infection by this bacterium has been associated with atherosclerosis, brain abscesses[111] and endocarditis[44].

Campylobacter rectus	PATHOGEN	Periodontitis associated pathogen

Gram-negative bacteria recognised as a major pathogen in periodontal disease. Together with other oral pathogens, *C. rectus* is implicated in adult periodontitis and rapidly advancing periodontitis[112]. *C. rectus* is often detected in larger numbers in subgingival pockets[113]. It is also associated with adverse pregnancy outcomes, including preterm delivery[37].

Tannerella forsythia	PATHOGEN	Major periodontitis pathogen

Alongside *Porphyromonas gingivalis, Tannerella forsythia* is widely regarded as a major pathogen. *T. forsythia* is an anaerobic, Gram-negative bacterium isolated from the gingival sulci and periodontal pockets of patients with periodontitis. Three pathogens *(T. forsythia, P. gingivalis, and T. denticola)*, known collectively as the "red complex", are strongly associated with the pathogenesis and progression of destructive forms of periodontitis. The presence of oral *T. forsythia* is associated with an increased risk of oesophageal cancer[114].

Prevotella spp. (P. intermedia, P. nigrescens)	COMMENSAL BACTERIA	Associated with periodontitis

Gram-negative bacteria implicated in various forms of human periodontal disease, including chronic periodontitis[115], early-onset periodontitis[6] and pregnancy gingivitis. *P. intermedia* and *P. nigrescens*, are members of the "orange complex", and are among the most frequently encountered species in subgingival plaque[116]. *P. intermedia* is a periodontitis-associated member of the subgingival microbiome[117]. Uzel et al. (2011) found that members of the green and orange complexes, such as *Prevotella intermedia*, increased much faster in periodontitis subjects than in periodontally healthy subjects[118].

Viral Pathogens

HSV-1	PATHOGEN	Causative agent of oral herpes

Herpes simplex virus 1 (HSV-1) is the causative agent of oral herpes. HSV-1 is a dsDNA virus that is prevalent in approximately 90% of the population. HSV-1 infection is often asymptomatic. Infection with HSV-1 may manifest as herpes lesions on the lips, gums and mouth. HSV-1 can also cause acute herpetic gingivostomatitis, a condition that results in ulcers of the mucous membranes inside the mouth. HSV-1 can be transferred via oral, vaginal and anal sex and through skin contact[119].

HPV-16_E6 & E7	PATHOGEN	Associated with oral cancer

Human papillomaviruses (HPV) are a large group of viruses, comprised of over 205 genotypes. HPV genotypes belonging to the *Alphapapillomavirus* group are of most clinical relevance as this group contains most of the mucosal HPVs, associated with cancer. HPV typically infects the basal cells of the epithelium[120] and can be transmitted through sexual activity and horizontal transmission and vertical transmission from mother to new born. HPV infection may be asymptomatic; a study of 4,000 subjects detected HPV in approximately 5% of individuals[121]. The HPV-16 genotype is the most common genotype to persist in the oral mucosa and is the most common genotype isolated from oral squamous cell carcinomas[26].

ORAL BIOMARKERS

Condition	ASSOCIATED WITH															
	Oral Disease	Anxiety	Depression	CRC	IBD	IBS	CFS	Diabetes	Metabolic Syndrome	Bacterial Endotoxemia (LPS)	Atherosclerotic Cardiovascular Disease	Obesity	Adverse pregancy outcomes	Cognitive	Oral cancer	Rheumatoid Arthritis
Periodontitis	x	x	x		x			x	x	x	x	x	x	x	x	x
Caries	x	x	x		x					x	x					
Oral Dysbiosis	x			x		x	x									

KEY	
x	Shows association with clinical condition. x does not represent causation.

DATA REFERENCES
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Sundararajan, 2015
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Winning, 2015
Fourie, 2016
Wang, 2018
Pussinen, 2007
Lin, 2016
Ye et al, 2016

SUMMARY

The Phylobioscience Oral EcologiX™ Oral Health and Microbiome Profile, is an innovative tool for analysis of the oral microbiota composition and host immune responses. Phylobioscience recommend the profile is used on an annual basis to monitor changes in oral health. The oral health and microbiome profile may also be used as a diagnostic tool in symptomatic patients.

For effective analysis of results, the interpretive guide should be used in combination with Invivo Healthcare's Oral Microbiome Clinical Considerations and the patient's symptomology and results.

The Oral Microbiome Clinical Considerations provides further information on lifestyle and environmental factors that may influence the oral microbiota. The Oral Microbiome Clinical Considerations also provides recommendations for treatment and management plans for results indicative of dysbiosis, inflammation, and infection.

If you have any queries on patient results or clinical considerations, please contact the Clinical Education team at **support@invivohealthcare.com**

RECOMMENDED READING

The oral microbiota: dynamic communities and host interactions

Lamont, R.J., Koo, H. and Hajishengallis, G., 2018.

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Dewhirst, F.E., Chen, T., Izard, J., Paster, B.J., Tanner, A.C., Yu, W.H., Lakshmanan, A. and Wade, W.G., 2010.

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Periodontal-disease-associated biofilm: A reservoir for pathogens of medical importance

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