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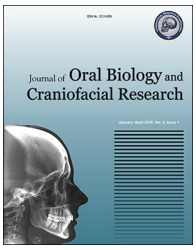

Review Article

Saliva as a diagnostic tool for oral and systemic diseases

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ABSTRACT

Early disease detection is not only vital to reduce disease severity and prevent complications, but also critical to increase success rate of therapy. Saliva has been studied extensively as a potential diagnostic tool over the last decade due to its ease and non-invasive accessibility along with its abundance of biomarkers, such as genetic material and proteins. This review will update the clinician on recent advances in salivary biomarkers to diagnose autoimmune diseases (Sjogren's syndrome, cystic fibrosis), cardiovascular diseases, diabetes, HIV, oral cancer, caries and periodontal diseases. Considering their accuracy, efficacy, ease of use and cost effectiveness, salivary diagnostic tests will be available in dental offices. It is expected that the advent of sensitive and specific salivary diagnostic tools and the establishment of defined guidelines and results following rigorous testing will allow salivary diagnostics to be used as chair-side tests for several oral and systemic diseases in the near future.

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1. Introduction

Early diagnosis of diseases is crucial to prevent complications that could have a negative impact on a patient's quality of life. For instance, ovarian cancer, the fifth most common cancer and cause of death in females, has a 5-year-survival rate of 10% when detected at stage 4 in comparison to 93% if diagnosed at stage 1.[1] Similarly, type 2 diabetes, which affects 7% of the adult population, can be solely controlled by diet and change in lifestyle if the diagnosis is made earlier.[2] Furthermore, despite the regular screenings and check-ups, many diseases are undetected until a late phase where morbid symptoms become apparent. To overcome these challenges, researchers are unravelling biomarkers. These biomarkers include genetic material (e.g. DNA, RNA) and protein molecules that reflect the current physiological state of an individual and hence help scientists to better understand the underlying cause of a disease.[3] Over the years, studies have shown that alterations in human genetics can be detected by molecular diagnostics, and anomalies in nucleic acids and proteins present in the patient's body fluids such as blood, cerebrospinal fluid (CSF) and urine can be used as effective biomarkers for disease diagnosis.[4–6] However, many obstacles remain such as lack of

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definite biomarkers for specific diseases, absence of inexpensive sample collection methods incurring minimal discomfort, and paucity of accurate and portable detection systems.[3] Fortunately, some of these limitations can be overcome by analysing one's saliva. Due to its ease and non-invasive accessibility along with its abundance of biomarkers such as genetic material and proteins,[3] saliva has been studied extensively as a potential diagnostic tool over the last decade.[7]

2. Properties of saliva as a diagnostic fluid

Although the utility and advantages of saliva as a screening tool for cystic fibrosis has been identified in the early 1960s,[8] its full diagnostic potential was discovered three decades later when studies revealed distinct advantages of saliva over serum.[9,10] Like serum, saliva also contains hormones, antibodies, growth factors, enzymes, microbes and their products.[7,11] As shown in Fig. 1, many of these constituents enter saliva through blood via passive diffusion, active transport or extracellular ultra filtration.[7,12] Therefore, saliva can be seen in many cases as a reflection of the physiological function of the body.[13] There have been concerns about the use of saliva for diagnostic purposes due to its low concentration of analytes in comparison to blood.[14] However, with the advent of highly sensitive molecular methods and nanotechnology, this is no longer a limitation.[15] Saliva as a diagnostic tool should be sought due to a multitude of compelling reasons summarized in Table 1. All these characteristics make saliva an appealing diagnostic candidate for the detection and monitoring of several biomarkers in infants, children, adults and uncooperative patients.[16]

Table 1 – Advantages of salivary testing for diagnosis.

Advantages[3,69–72]
Non-invasive, easy to use, inexpensive
Safer to administer than serum sampling (no needles)
Real-time diagnostic values
No need for trained medical staff
Multiple samples can be obtained easily
Collection and screening can be done at home
Minimal risks of cross-contamination
More economical sampling, shipping and storage compared to serum
Requires less manipulation during diagnostic procedures compared to serum
Commercial availability of screening assays

3. Autoimmune disorders

3.1. *Sjogren's syndrome*

Sjogren's syndrome (SS) is a chronic autoimmune disease characterized by salivary and lacrimal dysfunction, multiple organ abnormalities and serological changes.[17] Salivary secretions from these patients exhibit elevated levels of antibodies and cytokines such as IgA, IgG, prostaglandin-E2, and interleukin-6. This is accompanied by a reduction in oral phosphate levels and xerostomia due to reduced salivary flow, which can lead to infections, progressive caries, dysphagia and oral pain.[18] Current tests for SS include sialometry or salivary flow rate determination, salivary scintigraphy, sialography, serological tests or minor salivary gland biopsies. Although useful, these tests are invasive, expensive or in many cases non-conclusive.[17,19] Salivary proteomics represent a valuable tool to diagnose SS. It is based on the detection of several biomarkers that are simultaneously influenced by the disease. Recently, a panel of candidate salivary biomarkers of SS was investigated.[20] Twenty-eight proteins were found to be significantly modified by SS. The authors concluded that these tests, when performed on whole saliva, can diagnose SS, although larger clinical trials are warranted before they are brought to the market. Recently, salivary proteomics have gained attention with their advanced proteins for diagnoses, classification and/or predicting the prognosis of SS. Although saliva proteomics could provide new insights, however, still several questions remained unanswered. A study found salivary proteomics such as pSS biomarker as a potential

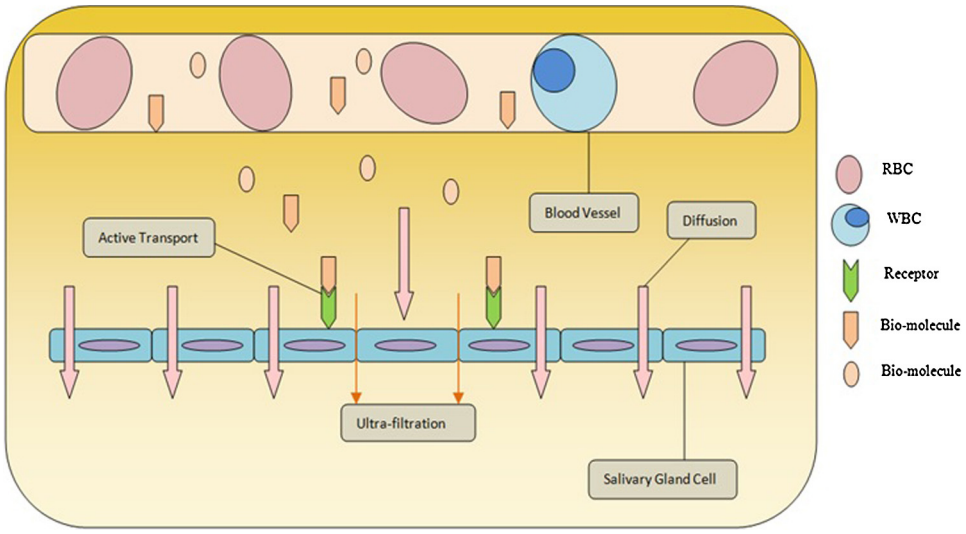


Fig. 1 – Schematic diagram illustrating key routes through which serum molecules enter saliva. This movement of constituents makes saliva functionally equal to serum for potential diagnosis of various diseases.

marker for the diagnosis of SS. These biomarkers are said to be associated with pathology, minor salivary glands and inflammation to an extent.[69]

3.2. *Cystic fibrosis*

Cystic fibrosis (CF), one of the most frequent hereditary disease in Caucasians, typically leads to early death from respiratory complications.[21] Mutations in the CF transmembrane conductance regulator protein is suspected to be involved in the chronic inflammation process occurring in the lungs of affected patients.[22] Saliva of CF patients has increased levels of calcium and phosphate, which may explain higher incidence of calculus observed in such individuals.[23] These patients also harbour higher salivary levels of chloride, potassium and sodium ions with a lower salivary volume and pH compared to healthy individuals.[24] In addition, whole saliva samples in younger CF patients have been found to have higher levels of proteins, antioxidants and uric acid compared to controls.[25] All these salivary changes are thought to be related to the chronic oxidative and inflammatory process activity in the oral cavity of these patients and represent biomarkers that could give more clues about the aetiology and monitoring of CF.

4. Cardiovascular diseases

Cardiovascular diseases are the leading cause of death in Canada.[26] Atherosclerosis, the leading etiological factor, is triggered by the presence of inflammation,[27,28] which results in deposition of lipids in the arterial walls and progressive narrowing of the arterial lumen. This condition might then culminate in acute myocardial infarction (AMI), a common lethal cardiovascular complication. A significant number of patients suffering from heart disease lack known risk factors such as family history, hypertension and increased lipid profiles.[29] Similarly, unlike subjects with high serum cholesterol levels, people with increased C-reactive protein (CRP) are more likely to be unaware of their susceptibility to develop cardiovascular disease.[30] CRP is an inflammatory mediator that is produced in response to acute injury or infection and can mediate an inflammatory response by triggering the complement cascade. It can contribute to atherogenesis and its presence has been demonstrated in arterial plaque.[31] Importantly, salivary CRP levels were found to correlate with plasma CRP levels obtained from blood samples of a population at risk for cardiovascular complications.[32] It is also possible to detect cardiac troponin (cTn), a biomarker for the detection of AMI in saliva that is released in response to cardiac cell necrosis.[33] Salivary cTn levels were shown to be a monitoring/diagnosis tool as sensitive as their serum levels in patients suffering from AMI.[34] There is little doubt that salivary tests will progressively replace blood samples to isolate several biomarkers associated with cardiovascular diseases.

5. Diabetes

From 1998-99 to 2008-09, the prevalence of diagnosed diabetes among Canadians has increased by 70%.[35] Common complications of diabetes involve multiple organs and include cardiovascular and periodontal diseases. Very little research has been done on salivary testing for the diagnosis of diabetes. This is most likely because easy-to-use pinprick tests are already available on the market to assess glucose blood levels. However, salivary proteomics offer an interesting option for those who prefer a less invasive approach for screening. A recent study reported the salivary proteomic profile of type 2 diabetes patients.[36] The authors found that 52 proteins were differently expressed and higher levels of some diabetes-related inflammatory biomarkers were observed in saliva of individuals with diabetes compared to controls. Other investigators have reported that among a total of 487 analysed proteins in the saliva, 65 had higher levels in type-2 diabetes subjects compared to healthy individuals.[37] Therefore, protein profiling in saliva could be an interesting avenue to diagnose and monitor diabetes in the future.

6. HIV

Human immune deficiency virus (HIV) affects the immune system and is integrated in the very genome of cells it attacks. It is a sexually-transmitted disease that also spreads through infected blood transfusions and from diseased mothers to infants.[38] Although there has been a slight decrease in the number of reported HIV-positive cases in Canada since 2008,[39] HIV virus transmission remains a concern due to the severe complications it can lead to if left untreated. In 2012, OraSure Technologies, Inc. (Bethlehem, PA) announced that the U.S. FDA had approved the over-the-counter OraQuick® In-Home HIV Test to detect both HIV-1 and HIV-2 viruses with an oral swab. The results can be obtained in the comfort of the user's home. A swab is left in place for 2–5 min between the lower gingival and buccal mucosa to collect antibodies in the saliva. Then, the swab is shipped to a predetermined laboratory for Western blot analysis to confirm the diagnosis. The reported specificity and sensitivity of this test are 99.98% and 93.0%, respectively, compared with the laboratory analysis using blood samples. [40,70] While the positive predictive value (proportion of positive results that are truly positive) of OraQuick® In-Home HIV Test of 98.7% is comparable to blood-based specimens in populations with a high prevalence of HIV, it drops to 88.6% in low prevalence populations.[41] This test is not available in Canada at this time.

7. Oral cancer

Oral squamous cell carcinoma (OSCC) is the most common form of oral cancer. The key to decrease OSCC mortality and morbidity is early detection. However, in asymptomatic patients, there is insufficient evidence to determine whether a visual and tactile oral screening test,[42] or commercially-available oral cancer screening methods (such as autofluorescence, tissue reflectance or transepithelial cytology)[43] will prevent oral cancer-related mortality. Therefore, other non-invasive screening options are needed.

Several research groups have found that salivary levels of specific proteins are increased in whole saliva of patients with

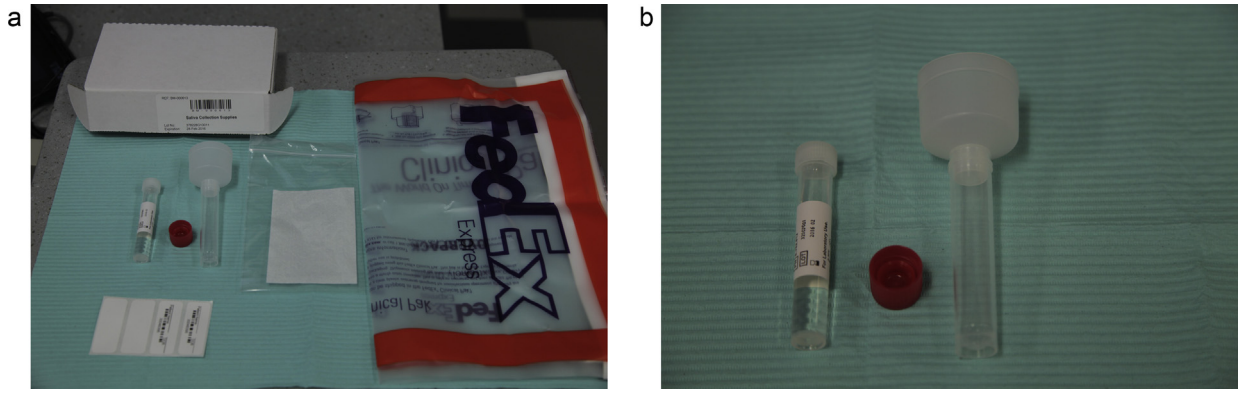


Fig. 2 – (a) Salivary test kit used to detect presence of HPV-16 associated with OSCC, levels of periodontal pathogens, and/or to determine genotypic status of IL-6 associated with periodontitis (Oral DNA® Labs, Eden Prairie, U.S.A.). (b) Sterile tube filled with saline on left. After swishing for 30 s, the patient spits in the tube with the funnel on right. The funnel is then unscrewed and the red cap, once screwed on, will seal the collection tube. It is then identified and shipped by priority mail for analysis.

OSCC. For example, CD44 (a cell surface glycoprotein involved in cell-to-cell interaction),[44] Cyfra 21-1 (a fragment of cytokeratin 19), tissue polypeptide antigen (TPS), and Cancer antigen 125 (CA-125) have been suggested as oral cancer biomarkers.[45] However, no single biomarker has been able to detect earlier stages of OSCC with accuracy, suggesting that only a panel of selected biomarkers could exhibit enough sensitivity to identify OSCC. The use of 7 OSCC-associated saliva RNAs (transcriptomes) has shown a prediction accuracy rate of 81%, demonstrating their potential as biomarkers for oral cancer detection.[46] A recent case-control study of 395 subjects validated 7 transcriptomes and 3 proteins as biomarkers for OSCC.[47] The increase in salivary levels of IL-8 and subcutaneous adipose tissue (SAT) demonstrated the highest levels of sensitivity and specificity to detect OSCC. Another significant biomarker for OSCC is the presence of human papillomavirus (HPV). A test is commercially available in U.S. and Canada for identifying individuals who are at risk of developing OSCC (OraRisk® HPV test, Oral DNA® Labs, Eden Prairie, U.S.A.) on the premise that 60% of OSCC tumours are associated with HPV-16 (Figs. 2a, b and 3).[48] Salivary biomarkers represent, therefore, a strong potential to isolate individuals who might develop oral cancer.

8. Oral diseases

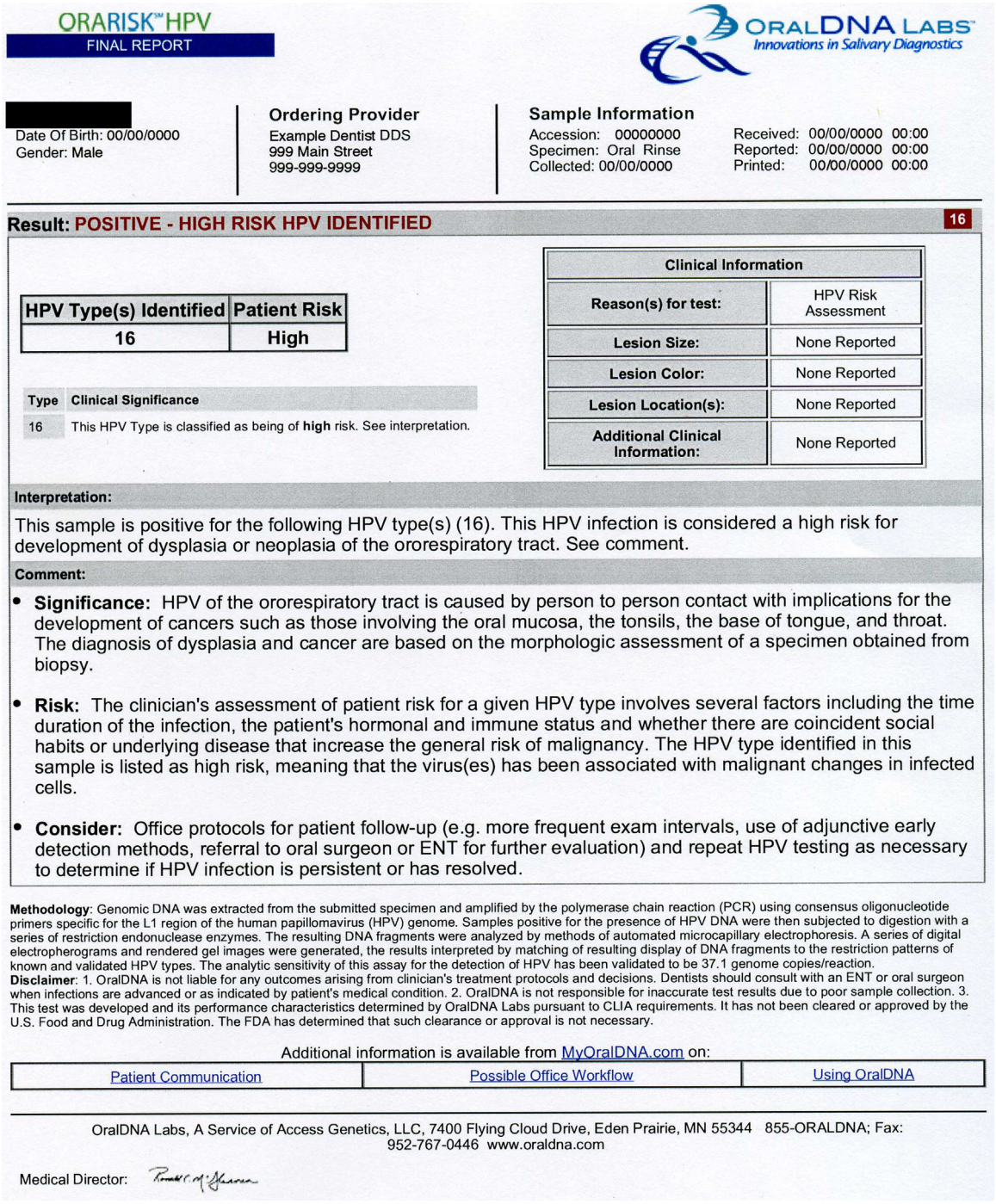
8.1. *Caries*

According to the Canadian Health Measures Survey (2007–09), 96% of Canadians have at least one or more decayed, missing, or filled teeth.[49] Caries is a result of demineralization of the tooth surface initiated by acid production of cariogenic bacteria.[50] This process can ultimately lead to tooth loss. Many studies have demonstrated the role of *S. mutans* in initiating dental caries, while *Lactobacilli* have a role in the progression of carious lesions.[51] High salivary levels of both pathogens using a commercially available test (CRT bacteria®, Ivoclar-Vivadent Inc., Amherst, U.S.A.) have shown a positive association with the presence of caries in children and adults.[52,53] On the other hand, saliva is known to play a protective role against caries since it contains several antibacterial agents, can mechanically clear the pathogens and has a buffering capacity to decrease the acid concentration on tooth surfaces.[54] Therefore, changes in quantity and composition of saliva can also provide potential tools to detect and monitor caries. However, no single salivary test has shown consistent accuracy in detecting caries. What has been suggested is rather a combination of known risk factors to predict individuals at risk for caries. However, none of the risk assessment programs proposed to date have shown consistent validity.[55] This can be explained by the involvement of multiple local and systemic risk factors in the caries development process.

8.2. *Periodontal diseases*

It was recently estimated that 47% of the population has periodontitis.[56] Among individuals diagnosed with periodontitis, 38% would have the moderate or severe form.[56] Like caries, periodontitis can lead to tooth loss.[57] It is characterized by the destruction of the periodontal tissues such as gingiva and bone that support the tooth. The activation of inflammatory mediators of host cells upon exposure to periodontal pathogens and their products primarily cause this condition.

Various salivary biomarkers have been studied for the diagnosis and prognosis of periodontal diseases. These include inflammatory mediators, enzymes, epithelial keratins, immunoglobulins, salivary ions and hormones.[58] Both whole saliva and gingival crevicular fluid (GCF) have been used in periodontics to detect these potential biomarkers. More specifically, the presence of matrix metalloproteinase-8 (MMP-8, an enzyme responsible for tissue destruction) in GCF has been positively associated with periodontitis progression.[59–61] In 2010, an immunochromatographic chair-side dipstick test became commercially available to determine the



ORARISK™ HPV
FINAL REPORT

ORALDNA LABS
Innovations in Salivary Diagnostics

[redacted]
Date Of Birth: 00/00/0000
Gender: Male

Ordering Provider
Example Dentist DDS
999 Main Street
999-999-9999

Sample Information
Accession: 00000000
Specimen: Oral Rinse
Collected: 00/00/0000
Received: 00/00/0000 00:00
Reported: 00/00/0000 00:00
Printed: 00/00/0000 00:00

Result: POSITIVE - HIGH RISK HPV IDENTIFIED 16

HPV Type(s) Identified	Patient Risk
16	High

Type	Clinical Significance
16	This HPV Type is classified as being **high** risk. See interpretation.

Clinical Information	
Reason(s) for test:	HPV Risk Assessment
Lesion Size:	None Reported
Lesion Color:	None Reported
Lesion Location(s):	None Reported
Additional Clinical Information:	None Reported

Interpretation:

This sample is positive for the following HPV type(s) (16). This HPV infection is considered a high risk for development of dysplasia or neoplasia of the ororespiratory tract. See comment.

Comment:

- **Significance:** HPV of the ororespiratory tract is caused by person to person contact with implications for the development of cancers such as those involving the oral mucosa, the tonsils, the base of tongue, and throat. The diagnosis of dysplasia and cancer are based on the morphologic assessment of a specimen obtained from biopsy.
- **Risk:** The clinician's assessment of patient risk for a given HPV type involves several factors including the time duration of the infection, the patient's hormonal and immune status and whether there are coincident social habits or underlying disease that increase the general risk of malignancy. The HPV type identified in this sample is listed as high risk, meaning that the virus(es) has been associated with malignant changes in infected cells.
- **Consider:** Office protocols for patient follow-up (e.g. more frequent exam intervals, use of adjunctive early detection methods, referral to oral surgeon or ENT for further evaluation) and repeat HPV testing as necessary to determine if HPV infection is persistent or has resolved.

Methodology: Genomic DNA was extracted from the submitted specimen and amplified by the polymerase chain reaction (PCR) using consensus oligonucleotide primers specific for the L1 region of the human papillomavirus (HPV) genome. Samples positive for the presence of HPV DNA were then subjected to digestion with a series of restriction endonuclease enzymes. The resulting DNA fragments were analyzed by methods of automated microcapillary electrophoresis. A series of digital electropherograms and rendered gel images were generated, the results interpreted by matching of resulting display of DNA fragments to the restriction patterns of known and validated HPV types. The analytic sensitivity of this assay for the detection of HPV has been validated to be 37.1 genome copies/reaction.
Disclaimer: 1. OralDNA is not liable for any outcomes arising from clinician's treatment protocols and decisions. Dentists should consult with an ENT or oral surgeon when infections are advanced or as indicated by patient's medical condition. 2. OralDNA is not responsible for inaccurate test results due to poor sample collection. 3. This test was developed and its performance characteristics determined by OralDNA Labs pursuant to CLIA requirements. It has not been cleared or approved by the U.S. Food and Drug Administration. The FDA has determined that such clearance or approval is not necessary.

Additional information is available from MyOralDNA.com on:

Patient Communication	Possible Office Workflow	Using OralDNA

OralDNA Labs, A Service of Access Genetics, LLC, 7400 Flying Cloud Drive, Eden Prairie, MN 55344 855-ORALDNA; Fax: 952-767-0446 www.oraldna.com

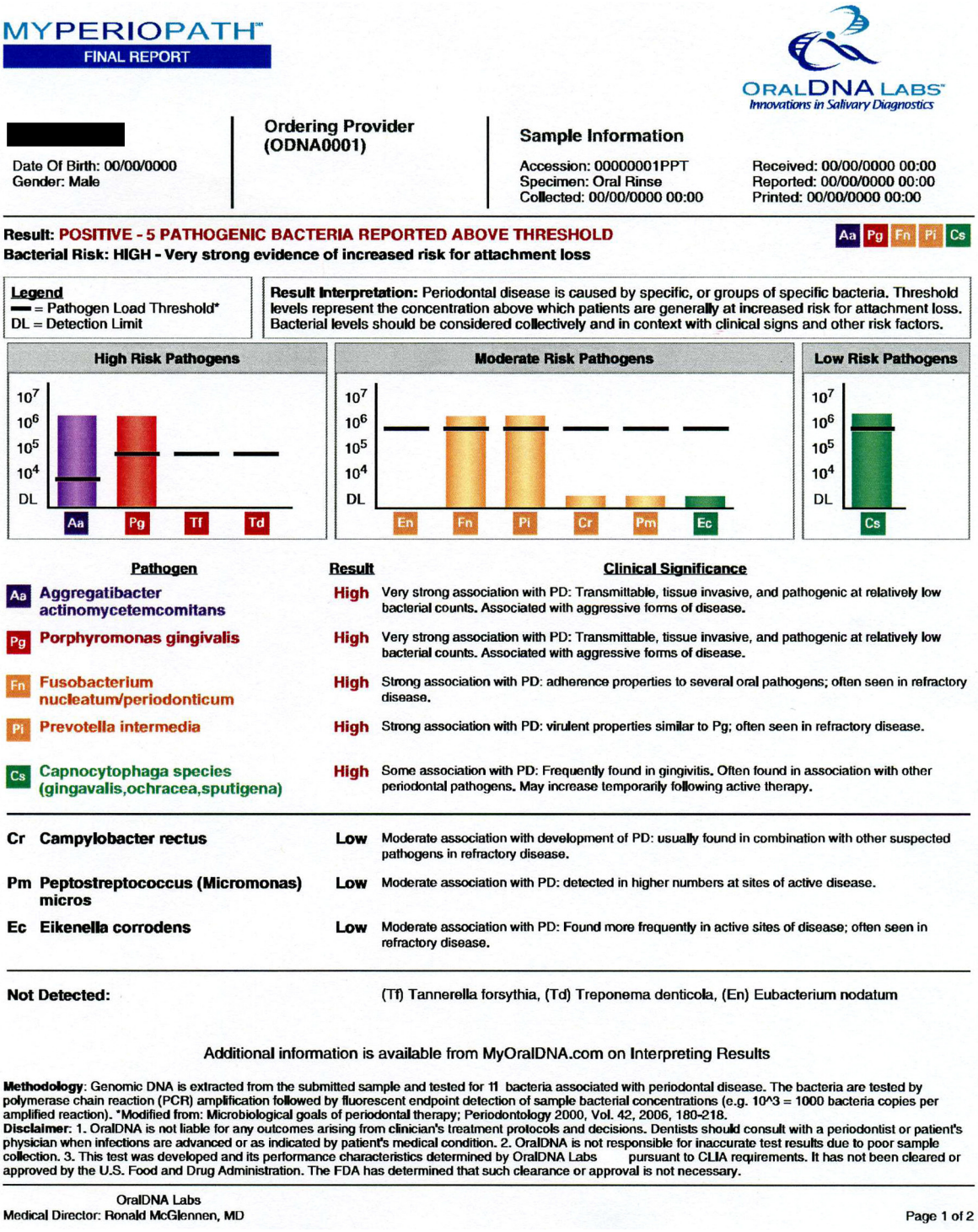
Medical Director:

Fig. 3 – Sample of a report for OraRisk® HPV test.
Reproduced with permission from Oral DNA® Labs.

presence or absence of MMP-8 in the GCF with similar precision as conventional laboratory assays.[62] Recently, it has been reported that salivary soluble toll-like receptor-2 and interleukin-4 correlate positively with periodontal disease process.[63] It has been proposed that not only host-derived factors should be analysed in the saliva but also oral pathogens to be able to predict periodontitis, since it is a multifactorial disease.[64] Indeed, investigators have found that higher salivary of levels of *Porphyromonas gingivalis*, *Tannerella forsythia* and *Prevotella intermedia* were found in individuals with progressive periodontitis.[65] This phenomenon has also been noted by a recent study, which shows that the combination of salivary *P. gingivalis* quantity with host specific pathogen response would be useful in diagnosing periodontitis with high accuracy.[71] A salivary test can detect most of the periodontal pathogens (MyPerioPath®, OralDNA® Labs). The

patient has to rinse with saline for 30 s then spit in a collection tube. The samples are then sent by priority mail to the laboratory for microbiological analysis. That test has been approved for chair-side use in the United States and Canada (Figs. 2a, b, 4a, and b).

Research has demonstrated that variations in more than 70 genes can be responsible for periodontal diseases.[66] Salivary genomics represent, therefore, another interesting avenue for the diagnosis of periodontitis. A patient's DNA obtained from saliva can be analysed at a designated laboratory for the genotypic status of interleukin-6 (IL-6), a cytokine involved in periodontal tissue destruction. It was recently validated that genetic mutations of the IL-6 gene are a significant risk factor for chronic periodontitis in Caucasians.[67] Such a test has been



MYPERIOPATH™
FINAL REPORT

ORALDNA LABS™
Innovations in Salivary Diagnostics

[redacted]
Date Of Birth: 00/00/0000
Gender: Male

Ordering Provider
(ODNA0001)

Sample Information
Accession: 00000001PPT
Specimen: Oral Rinse
Collected: 00/00/0000 00:00
Received: 00/00/0000 00:00
Reported: 00/00/0000 00:00
Printed: 00/00/0000 00:00

Result: POSITIVE - 5 PATHOGENIC BACTERIA REPORTED ABOVE THRESHOLD Aa Pg Fn Pi Cs
Bacterial Risk: HIGH - Very strong evidence of increased risk for attachment loss

Legend
▬ = Pathogen Load Threshold*
DL = Detection Limit

Result Interpretation: Periodontal disease is caused by specific, or groups of specific bacteria. Threshold levels represent the concentration above which patients are generally at increased risk for attachment loss. Bacterial levels should be considered collectively and in context with clinical signs and other risk factors.

Pathogen	Result	Clinical Significance
Aa Aggregatibacter actinomycetemcomitans	High	Very strong association with PD: Transmittable, tissue invasive, and pathogenic at relatively low bacterial counts. Associated with aggressive forms of disease.
Pg Porphyromonas gingivalis	High	Very strong association with PD: Transmittable, tissue invasive, and pathogenic at relatively low bacterial counts. Associated with aggressive forms of disease.
Fn Fusobacterium nucleatum/periodonticum	High	Strong association with PD: adherence properties to several oral pathogens; often seen in refractory disease.
Pi Prevotella intermedia	High	Strong association with PD: virulent properties similar to Pg; often seen in refractory disease.
Cs Capnocytophaga species (gingavalis,ochracea,sputigena)	High	Some association with PD: Frequently found in gingivitis. Often found in association with other periodontal pathogens. May increase temporarily following active therapy.
Cr Campylobacter rectus	Low	Moderate association with development of PD: usually found in combination with other suspected pathogens in refractory disease.
Pm Peptostreptococcus (Micromonas) micros	Low	Moderate association with PD: detected in higher numbers at sites of active disease.
Ec Eikenella corrodens	Low	Moderate association with PD: Found more frequently in active sites of disease; often seen in refractory disease.

Not Detected: (Tf) Tannerella forsythia, (Td) Treponema denticola, (En) Eubacterium nodatum

Additional information is available from MyOralDNA.com on Interpreting Results

Methodology: Genomic DNA is extracted from the submitted sample and tested for 11 bacteria associated with periodontal disease. The bacteria are tested by polymerase chain reaction (PCR) amplification followed by fluorescent endpoint detection of sample bacterial concentrations (e.g. 10^3 = 1000 bacteria copies per amplified reaction). *Modified from: Microbiological goals of periodontal therapy; Periodontology 2000, Vol. 42, 2006, 180-218.
Disclaimer: 1. OralDNA is not liable for any outcomes arising from clinician's treatment protocols and decisions. Dentists should consult with a periodontist or patient's physician when infections are advanced or as indicated by patient's medical condition. 2. OralDNA is not responsible for inaccurate test results due to poor sample collection. 3. This test was developed and its performance characteristics determined by OralDNA Labs pursuant to CLIA requirements. It has not been cleared or approved by the U.S. Food and Drug Administration. The FDA has determined that such clearance or approval is not necessary.

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Medical Director: Ronald McGlennen, MD

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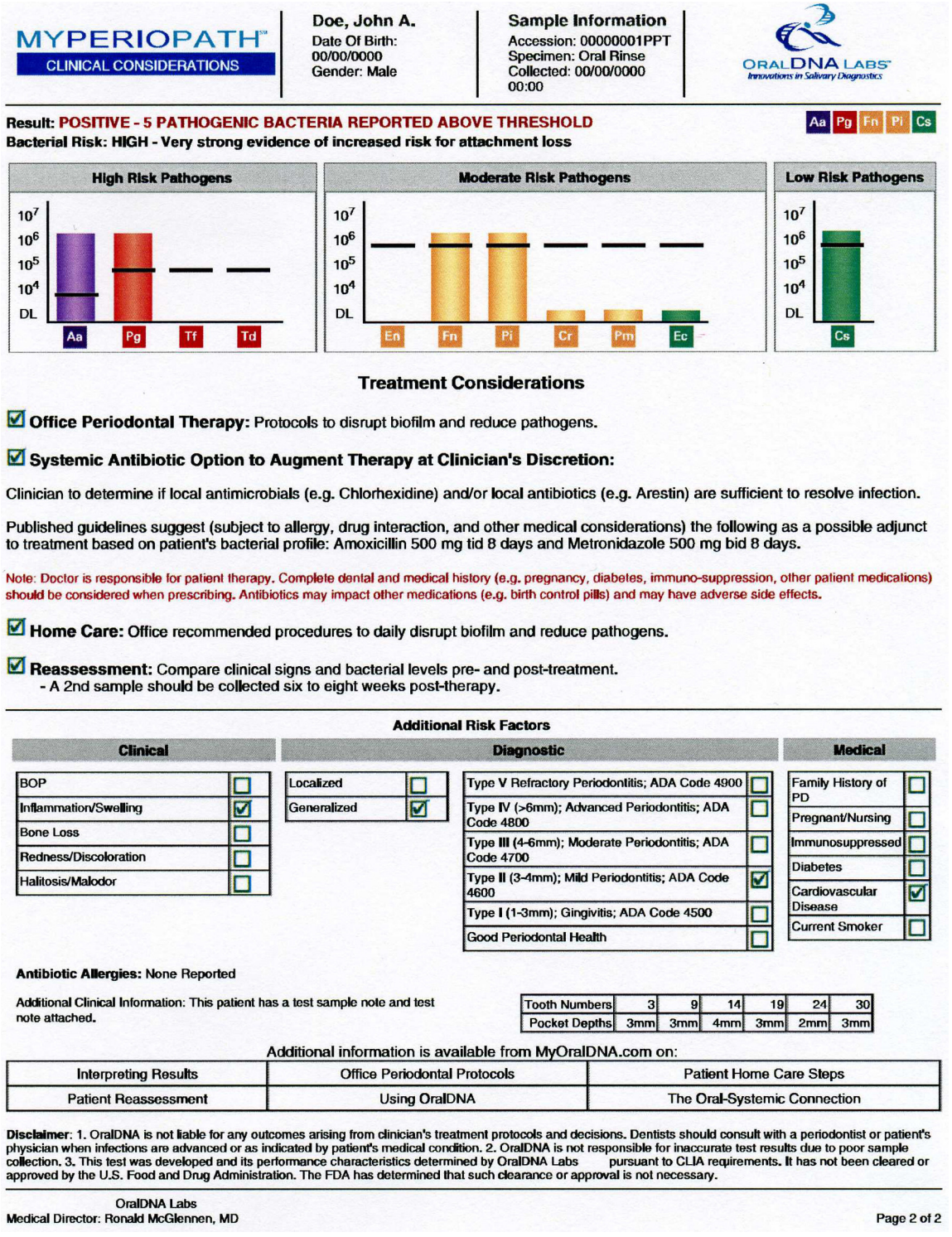
Fig. 4 – (a) and (b) Sample of a two-page report for MyPerioPath®. Reproduced with permission from Oral DNA® Labs.



MYPERIOPATH™
CLINICAL CONSIDERATIONS

Doe, John A.
Date Of Birth: 00/00/0000
Gender: Male

Sample Information
Accession: 00000001PPT
Specimen: Oral Rinse
Collected: 00/00/0000 00:00

ORALDNA LABS™
Innovations in Salivary Diagnostics

Result: POSITIVE - 5 PATHOGENIC BACTERIA REPORTED ABOVE THRESHOLD
Bacterial Risk: HIGH - Very strong evidence of increased risk for attachment loss

Aa Pg Fn Pi Cs

Treatment Considerations

☑ **Office Periodontal Therapy:** Protocols to disrupt biofilm and reduce pathogens.

☑ **Systemic Antibiotic Option to Augment Therapy at Clinician's Discretion:**

Clinician to determine if local antimicrobials (e.g. Chlorhexidine) and/or local antibiotics (e.g. Arestin) are sufficient to resolve infection.

Published guidelines suggest (subject to allergy, drug interaction, and other medical considerations) the following as a possible adjunct to treatment based on patient's bacterial profile: Amoxicillin 500 mg tid 8 days and Metronidazole 500 mg bid 8 days.

Note: Doctor is responsible for patient therapy. Complete dental and medical history (e.g. pregnancy, diabetes, immuno-suppression, other patient medications) should be considered when prescribing. Antibiotics may impact other medications (e.g. birth control pills) and may have adverse side effects.

☑ **Home Care:** Office recommended procedures to daily disrupt biofilm and reduce pathogens.

☑ **Reassessment:** Compare clinical signs and bacterial levels pre- and post-treatment.
- A 2nd sample should be collected six to eight weeks post-therapy.

Additional Risk Factors

Clinical		Diagnostic			Medical	
BOP	☐	Localized	☐	Type V Refractory Periodontitis; ADA Code 4900 ☐	Family History of PD	☐
Inflammation/Swelling	☑	Generalized	☑	Type IV (>6mm); Advanced Periodontitis; ADA Code 4800 ☐	Pregnant/Nursing	☐
Bone Loss	☐			Type III (4-6mm); Moderate Periodontitis; ADA Code 4700 ☐	Immunosuppressed	☐
Redness/Discoloration	☐			Type II (3-4mm); Mild Periodontitis; ADA Code 4600 ☑	Diabetes	☐
Halitosis/Malodor	☐			Type I (1-3mm); Gingivitis; ADA Code 4500 ☐	Cardiovascular Disease	☑
				Good Periodontal Health ☐	Current Smoker	☐

Antibiotic Allergies: None Reported

Additional Clinical Information: This patient has a test sample note and test note attached.

Tooth Numbers	3	9	14	19	24	30
Pocket Depths	3mm	3mm	4mm	3mm	2mm	3mm

Additional information is available from MyOralDNA.com on:

Interpreting Results	Office Periodontal Protocols	Patient Home Care Steps
Patient Reassessment	Using OralDNA	The Oral-Systemic Connection

Disclaimer: 1. OralDNA is not liable for any outcomes arising from clinician's treatment protocols and decisions. Dentists should consult with a periodontist or patient's physician when infections are advanced or as indicated by patient's medical condition. 2. OralDNA is not responsible for inaccurate test results due to poor sample collection. 3. This test was developed and its performance characteristics determined by OralDNA Labs pursuant to CLIA requirements. It has not been cleared or approved by the U.S. Food and Drug Administration. The FDA has determined that such clearance or approval is not necessary.

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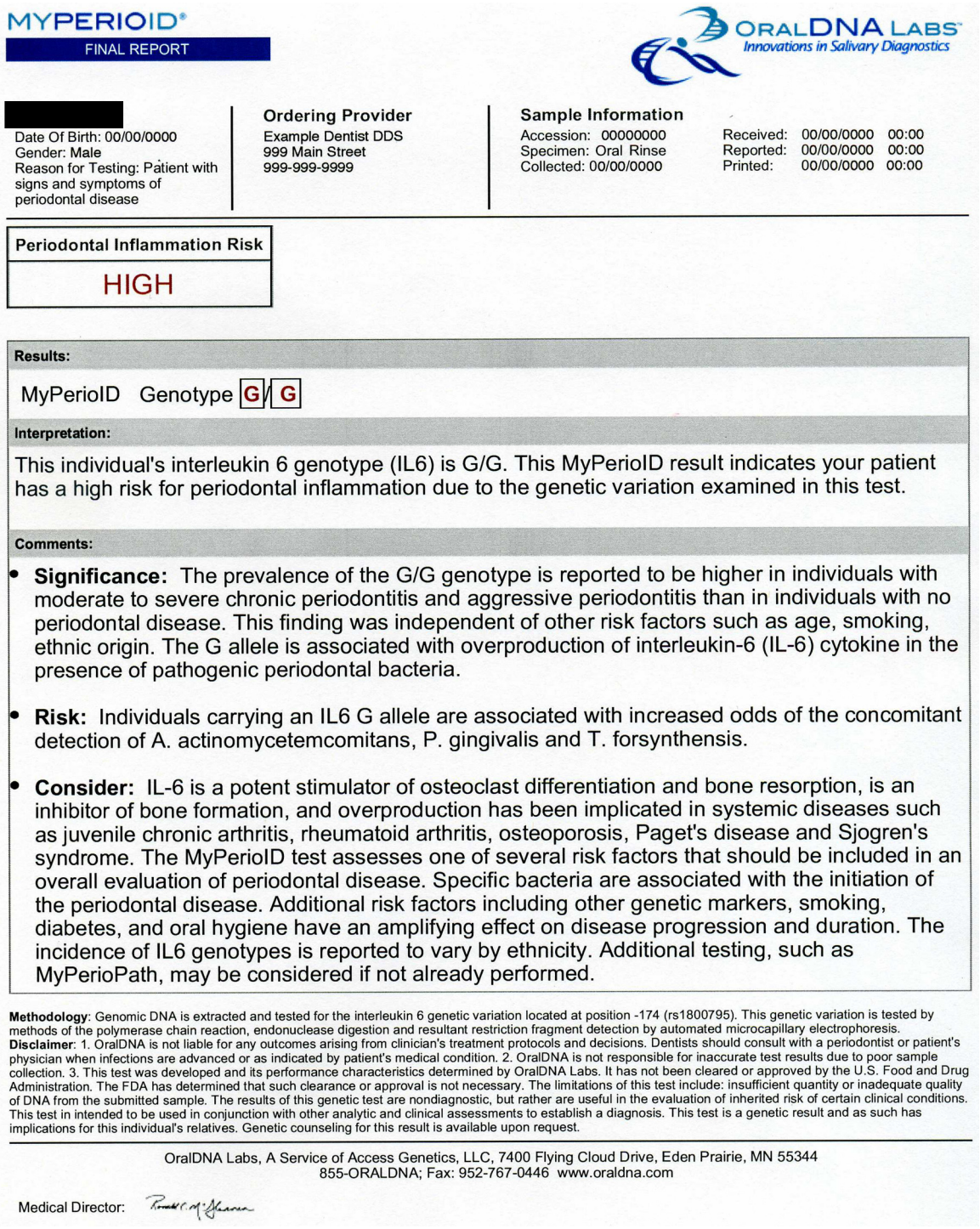
Fig. 4. (Continued).



MYPERIOID®
FINAL REPORT

ORALDNA LABS
Innovations in Salivary Diagnostics

[redacted]
Date Of Birth: 00/00/0000
Gender: Male
Reason for Testing: Patient with signs and symptoms of periodontal disease

Ordering Provider
Example Dentist DDS
999 Main Street
999-999-9999

Sample Information
Accession: 00000000
Specimen: Oral Rinse
Collected: 00/00/0000
Received: 00/00/0000 00:00
Reported: 00/00/0000 00:00
Printed: 00/00/0000 00:00

Periodontal Inflammation Risk
HIGH

Results:
MyPerioID Genotype G/G

Interpretation:
This individual's interleukin 6 genotype (IL6) is G/G. This MyPerioID result indicates your patient has a high risk for periodontal inflammation due to the genetic variation examined in this test.

Comments:
- **Significance:** The prevalence of the G/G genotype is reported to be higher in individuals with moderate to severe chronic periodontitis and aggressive periodontitis than in individuals with no periodontal disease. This finding was independent of other risk factors such as age, smoking, ethnic origin. The G allele is associated with overproduction of interleukin-6 (IL-6) cytokine in the presence of pathogenic periodontal bacteria.
- **Risk:** Individuals carrying an IL6 G allele are associated with increased odds of the concomitant detection of A. actinomycetemcomitans, P. gingivalis and T. forsynthensis.
- **Consider:** IL-6 is a potent stimulator of osteoclast differentiation and bone resorption, is an inhibitor of bone formation, and overproduction has been implicated in systemic diseases such as juvenile chronic arthritis, rheumatoid arthritis, osteoporosis, Paget's disease and Sjogren's syndrome. The MyPerioID test assesses one of several risk factors that should be included in an overall evaluation of periodontal disease. Specific bacteria are associated with the initiation of the periodontal disease. Additional risk factors including other genetic markers, smoking, diabetes, and oral hygiene have an amplifying effect on disease progression and duration. The incidence of IL6 genotypes is reported to vary by ethnicity. Additional testing, such as MyPerioPath, may be considered if not already performed.

Methodology: Genomic DNA is extracted and tested for the interleukin 6 genetic variation located at position -174 (rs1800795). This genetic variation is tested by methods of the polymerase chain reaction, endonuclease digestion and resultant restriction fragment detection by automated microcapillary electrophoresis.
Disclaimer: 1. OralDNA is not liable for any outcomes arising from clinician's treatment protocols and decisions. Dentists should consult with a periodontist or patient's physician when infections are advanced or as indicated by patient's medical condition. 2. OralDNA is not responsible for inaccurate test results due to poor sample collection. 3. This test was developed and its performance characteristics determined by OralDNA Labs. It has not been cleared or approved by the U.S. Food and Drug Administration. The FDA has determined that such clearance or approval is not necessary. The limitations of this test include: insufficient quantity or inadequate quality of DNA from the submitted sample. The results of this genetic test are nondiagnostic, but rather are useful in the evaluation of inherited risk of certain clinical conditions. This test in intended to be used in conjunction with other analytic and clinical assessments to establish a diagnosis. This test is a genetic result and as such has implications for this individual's relatives. Genetic counseling for this result is available upon request.

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Fig. 5 – Sample of a report for MyPerioID®.
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used in the United States and Canada (MyPerioID®, OralDNA® Labs) (Figs. 2a, b and 5). There is little doubt that future research to isolate genetic, microbiological and host-derived risk factors will shed more light on potential biomarkers for periodontal diseases.

9. Conclusion

Considering their accuracy, efficacy, ease of use and cost effectiveness, salivary diagnostic tests have demonstrated

their applications in clinical and basic sciences. Moreover, salivary-based diagnostic techniques can potentially allow screening of an entire population for a specific disease in a timely fashion. Given that patients visit their dentists more frequently than their physicians, it has been suggested that salivary tests will pave the way for chair-side diagnosis of multiple oral and systemic diseases at the dental office.[68] However, much work needs to be done to incorporate saliva-based diagnostics into daily use. Salivary collection methods and biomarkers need to be standardized and validated. Also, new assays and devices need to be developed at a commercially feasible rate. This can incur significant cost and may require cooperative agreement between different stakeholders including the government, funding agencies, academia and private sector. Last but not least, such non-traditional, saliva-based diagnostic tests would require general acceptance by insurance companies, dentists and other health care professionals, for which further studies need to demonstrate and establish their accuracy and cost effectiveness. It is expected that the advent of sensitive and specific salivary diagnostic tools and the establishment of defined guidelines will make salivary diagnostics a reality in the near future.

Conflicts of interest

The authors have none to declare.

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