A Practical Guide to Salivary Diagnostics

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AAOSH
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Overview of Presentation

- Bringing the clinical laboratory to the dental profession
- Applications of molecular genetics in the dental practice
  - Evaluating oral pathogens in periodontitis
  - Genetic risk assessment for CP and systemic health
- Towards a more comprehensive program in screening for oral cancer

Introduction

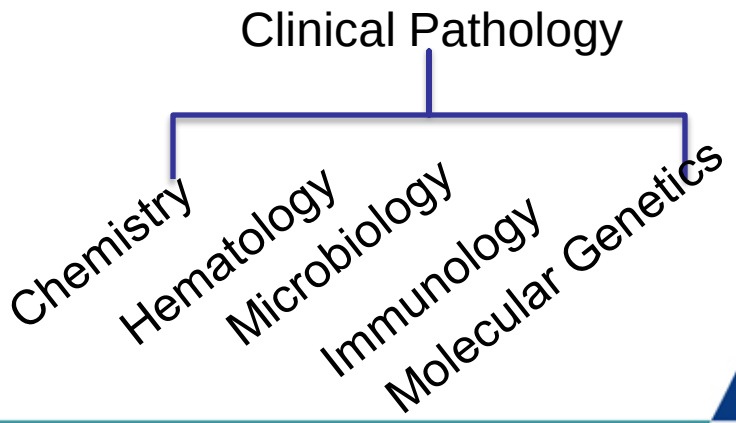
- Saliva is an obvious choice for clinical lab testing:
  - Easy to collect
  - Not, very, offensive
  - Representative of the state of health and disease in:
    - The ororespiratory tract
    - A source of genetic material
    - A reflection of oral AND systemic health

Saliva to Screen for Hyperglycemia

- Saliva is a transudate of serum
  - As such saliva is a poor source of free hemoglobin, or other serum proteins
- Because of this measuring these analytes in saliva have a low sensitivity and hence a poor predictive value
- Future tests, however, will leverage the analysis of saliva specific proteins
  - Stay tuned for tests such as glycosylated lysozyme!

Organization of the Clinical Laboratory

- The clinical lab has 2 areas of specialization:
  - Anatomic Pathology
  - Clinical Pathology
- Anatomic pathology deals with cells and tissue.
  - Biopsies and diagnoses made with a microscope
- Clinical pathology tests for all things from body fluids
  - Chemistry, microbiology, hematology
  - Molecular or genetic testing fits under this category



OralDNA Labs: Mission

- Providing healthcare professionals with tests to prevent, diagnose and prognose oral and systemic disease
- Oral DNA tests are:
  - Objective
  - Reliable
  - Clinically useful and practical
- The remainder of the lecture will details the portfolio of salivary diagnostic tests available today

Laboratory Testing for Periodontitis

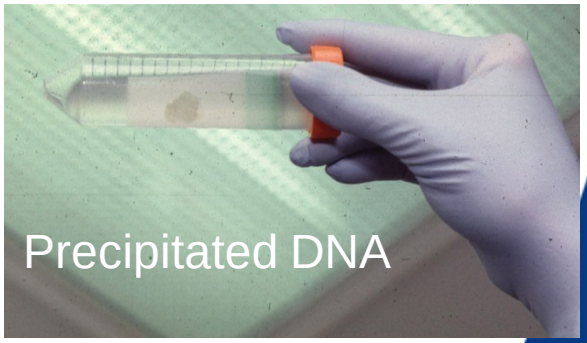
- The etiology of chronic periodontitis (CP) is multifactorial, including
  - the effect of pathogenic bacteria causing infection
  - the host's genetic disposition
  - the effect of coincident medical, environmental, and behavioral factors
- The advent of clinical laboratory services for the dental profession provides objective and actionable information to:
  - prevent
  - diagnose
  - prognose and management of CP

Laboratory Tools to Assess Periodontitis

- Conventional approaches to assess chronic periodontitis include:
  - Microbial culture
  - Chemical analysis of GCF and Saliva
- Microbial culture has the advantage to capture the full spectrum of bacteria resident in the mouth
  - The problem is, there are so many bacteria
  - Challenges to support methods to isolate anaerobes and facultative anaerobes
  - Microbial culture not very specific
- Molecular genetic methods are both highly sensitivity and specific
  - Molecular tests can also be quantitative


Blood agar plate with mixed flora

Versus

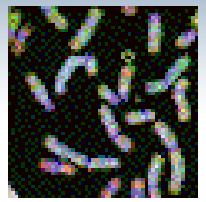

Precipitated DNA

The Human Genome: New Vocabulary

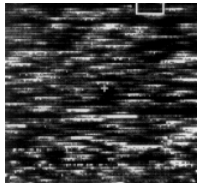
The Human Genome Project

- A $12 billion commitment to sequence $3x10^9$ bases
- Cloned cDNA derived from expression library of several species
- Leveraged acceleration of population genetics and linkage maps- tied strongly to industry champions
- New gene discovered answer questions about clinically overt disease. Education platform for the public makes genetics a household term

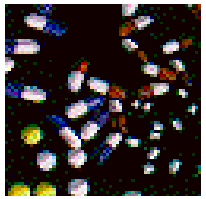
Genomics
- Sequencing the human genome
- New gene discovery



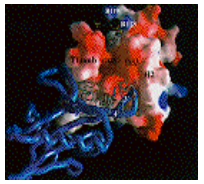
Gene Expression
- Sequencing the human genome
- New gene discovery



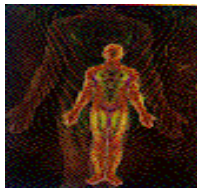
Pharmacogenomics
- Sequencing the human genome
- New drug discovery
- Applied to specific people is pharmacogenetics



Structural Genomics
- Derived structure of proteins form knowledge of the sequence of expressed genes



Diagnostics
- New disease genes characterize
- New technology for routine clinical use



Functional Genomics
- Derived knowledge of protein structure
- Deduced knowledge of protein and subcellular system function

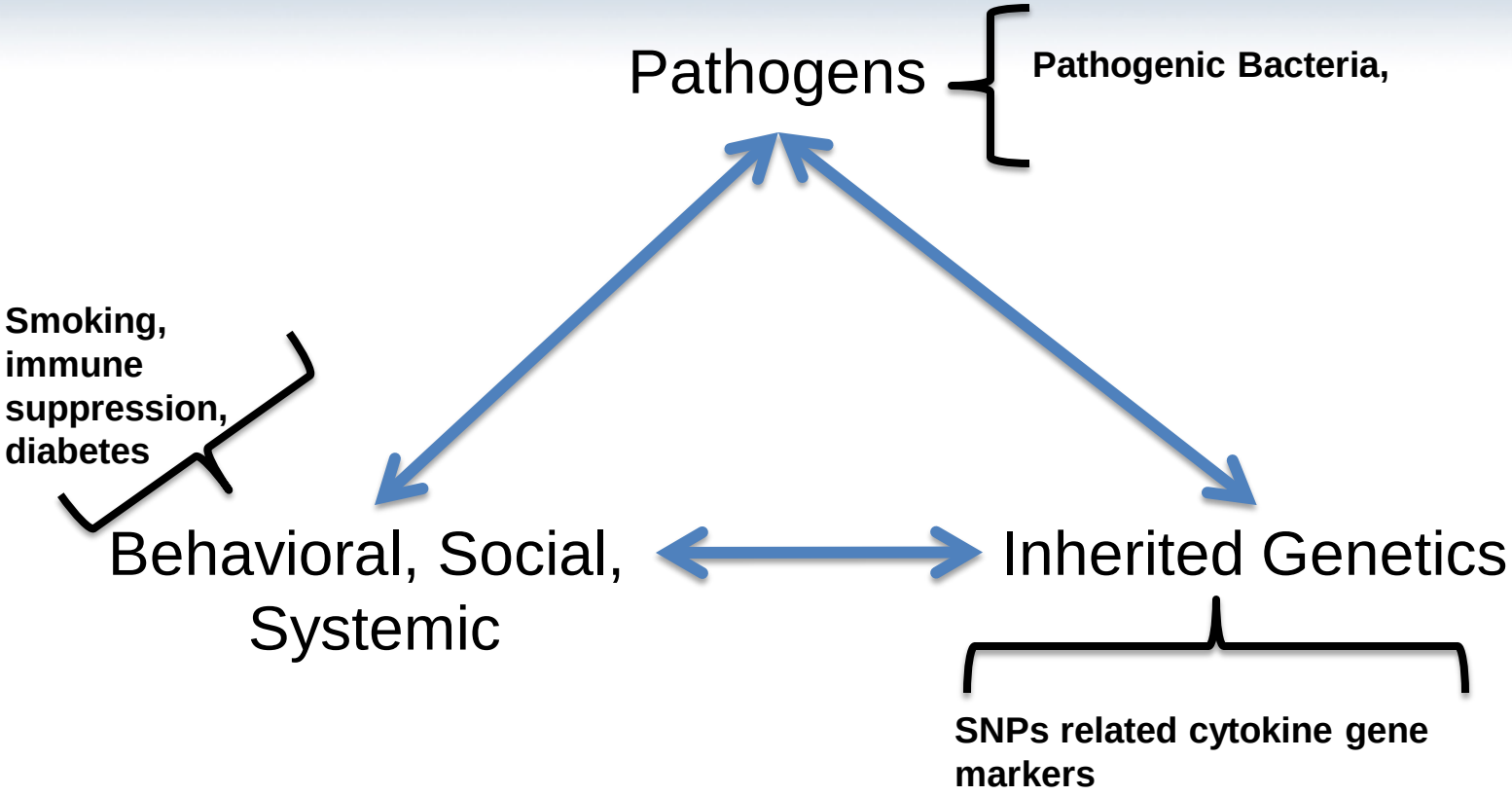
Molecular Diagnostics in the Management of Periodontal Disease

- Molecular diagnostics uses DNA and RNA to diagnose and characterize disease

- The types of tests performed in molecular diagnostics include:
  - Infectious disease
  - Inherited disease
  - Acquired disease such as cancer
  - ✓ Genetic susceptibility

- Molecular tests are available today to aid in the management of periodontitis

Modeling the Causes of Periodontitis



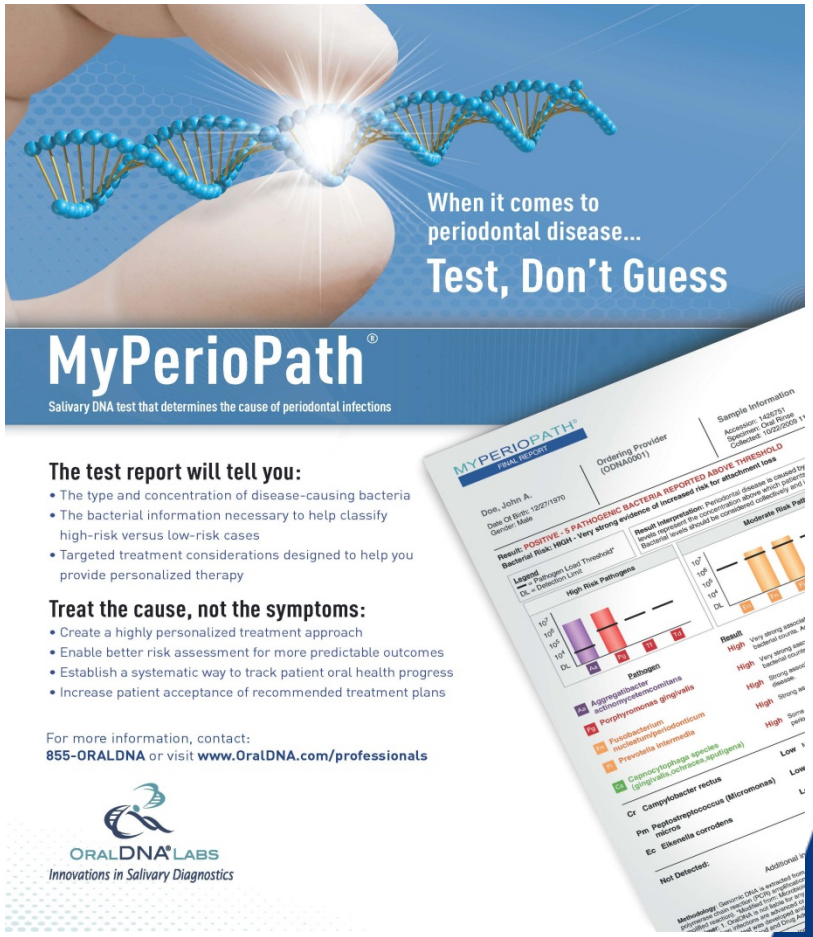
The current model employs the clinical lab to test for oral pathogens and human genomic DNA to test for inherited markers linked to disease risk, while the clinician provides the input of the patient demographics

Molecular Detection and Quantification of Microbial Pathogens

- Assaying for the pathogenic bacteria helps to support the clinical diagnosis of periodontitis
  - From the collected sample, bacterial and human genomic DNA is extracted
  - PCR is employed to amplify, specifically the consort of microbes
- With positive, negative and quantitation controls the result of each test will:
  - Measure the type and amounts of the pathogens
  - Be used to assess the effect before and after therapy
  - Guide in the selection of the most effective therapies

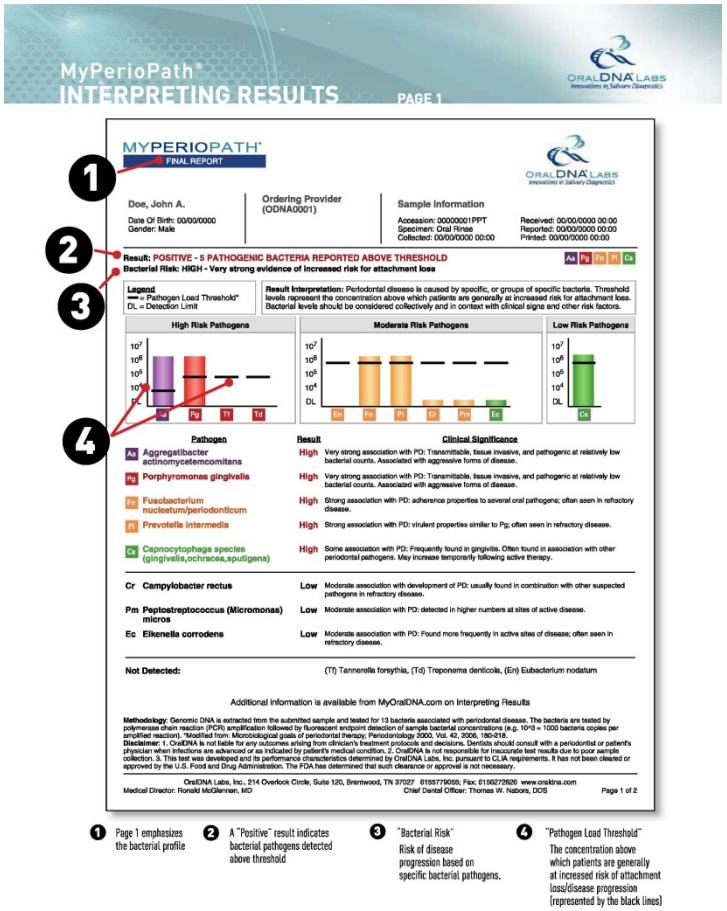
MyPerioPath®: Quantitative Detection of Periodontal Bacteria

- MyPerioPath®, or MPP measures the types and amount of 13 pathogenic bacteria associated with chronic periodontitis
  - Sample is an oral rinse
  - Results are displayed as histograms in color
  - The results are compared to therapeutic threshold
- MyPerioProgress™ is a format to compare before and after results



How to Read and Understand the MyPerioPath® Report

1. Final report is ready to present to the patient
2. Positive or Negative refers to whether there are any bacterial measured over the therapeutic threshold
3. Risk assessment refers to what category of bacteria are detected above threshold
4. Graphical display show which bacteria are detected and their amount relative to the therapeutic threshold



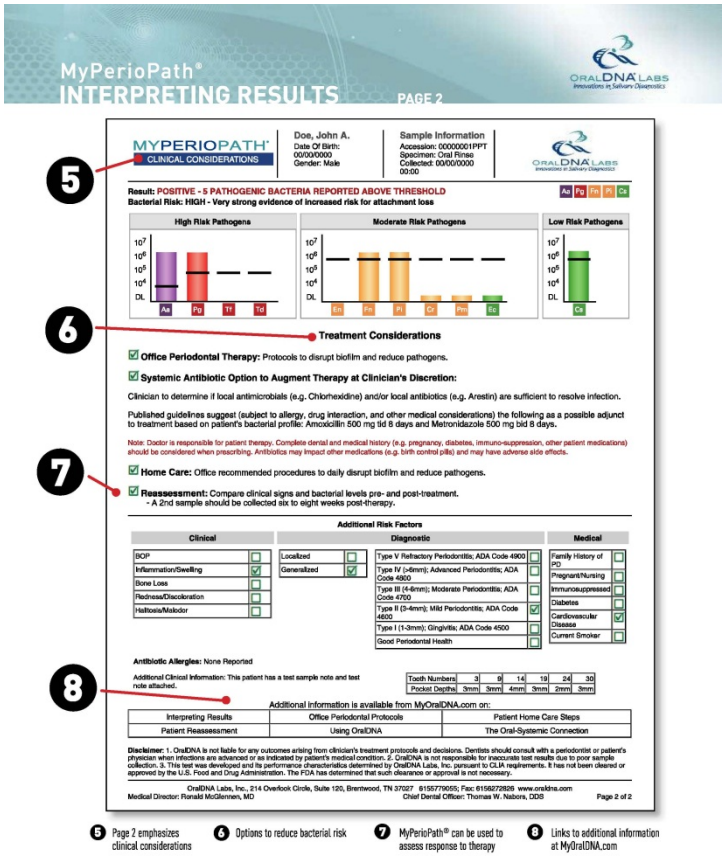
How to Read and Understand the MyPerioPath® Report

5 Second page contains information on clinical interpretation

6 Listing of “treatment considerations”

7 Emphasis on the indications and timeframe for following testing upon completion of therapy

8 Links to more information



Value in Taking A Thorough History

Collected demographic information on more than 50,000 submitted samples

Authors	Subjects, *N*	Time period	Prognostic factors	Outcome variable for disease progression
Barr et al. (1992)	114 men 86 HIV+; 28 HIV −	20 months	Immunosuppression	Attachment loss
Yeung et al. (1993)	30 HIV+; 10 HIV −	18 months	HIV	Attachment loss
Kaldahl et al. (1996)	74	72 months	Smoking	Attachment loss
McGuire & Nunn (1996)	100	⩾60 months	Smoking	Tooth loss
Krall et al. (1997)	1225	6 years	Smoking	Tooth loss
Machtei et al. (1997)	79	12 months	Smoking, *T.f, P.i, P.g*, mean baseline AL	Bone loss
Boström et al. (1998)	57	60 months	Smoking	Bone loss
Taylor et al. (1998)	362	2 years	Diabetes	Bone loss
McGuire & Nunn (1999)	42	⩾60 months	Smoking, IL-1 positive	Tooth loss
Norderyd et al. (1999)	361	10 years	Smoking	Bone loss
Payne et al. (1999)	38 (non-smokers)	2 years	Osteoporosis	Bone loss
Bergström et al. (2000)	101	10 years	Smoking	Bone loss
De Sanctis & Zuchelli (2000)	40 (32 non-smokers)	48 months	IL-1 positive	Attachment loss
Timmerman et al. (2000)	167 untreated	7 years	*A.a*	Attachment loss
Tran et al. (2001)	205	2 years	*T.f*	Attachment loss
Chen et al. (2001)	177 males	10 years	Smoking	Attachment loss/tooth loss
Nieri et al. (2002)	60 (non-smokers)	10 years	(IL-1 genotype × initial mean bone level)	Bone loss
Jansson et al. (2002)	507	20 years	Smoking	Bone loss
Ogawa et al. (2002)	394 (⩾70 years old)	24 months	Smoking, baseline AL ⩾6 mm	Attachment loss
Kamma & Baehni (2003)	25 (AP)	60 months	Smoking, stress, *P.g, T.d, N* of teeth lost	Attachment loss
Fardal et al. (2004)	100	9–11 years	Smoking	Tooth loss
Paulander et al. (2004)	259 (50 years)	10 years	Smoking, % approximal sites with PD ⩾4 mm *N* of teeth, systemic disease (diabetes, hormonal or cardiovascular disease)	Bone loss
Yoshihara et al. (2004)	179 (non-smokers) ⩾70 years	3 years	Osteopenia	Attachment loss

AP, aggressive periodontitis; AL, attachment level; *T.f, Tanerella forsythensis*; *P.i, Prevotella intermedia*; *P.g, Porphyromonas gingivalis*; *T.d, Treponema denticola*; IL, interleukin.

Smoking	- Bone Loss	+ Bone Loss
No	43.5%	56.52%
Yes	18.7%	81.3%

Smoking	-inflam.	+ Inflam.
No	40.9%	59.1%
Yes	27.9%	72.1%

Finding- a history of smoking is highly predictive of bone and attachment loss

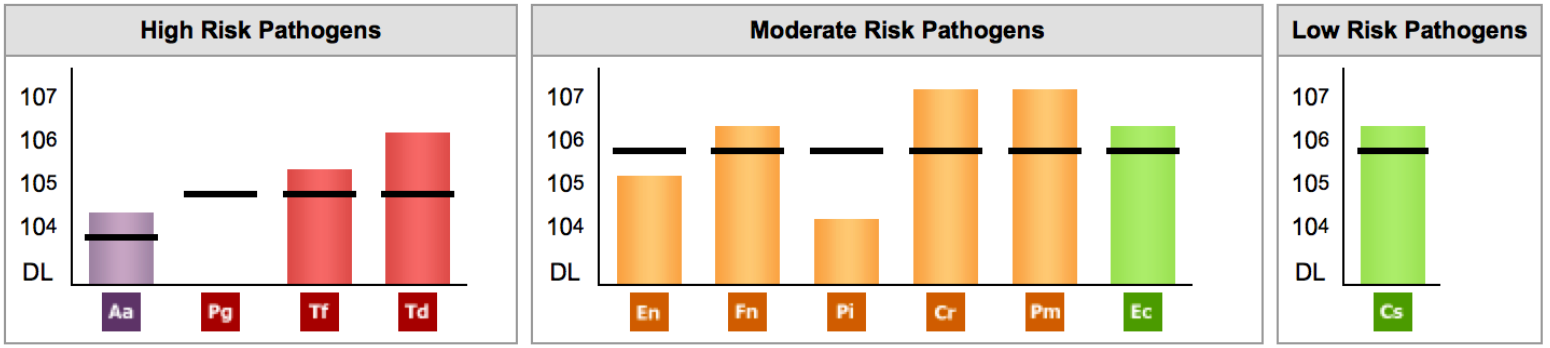
Periodontal Pathogen Testing Makes the Assignment of Disease Status Objective

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	☐	Immunosupressed	☐
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	☐

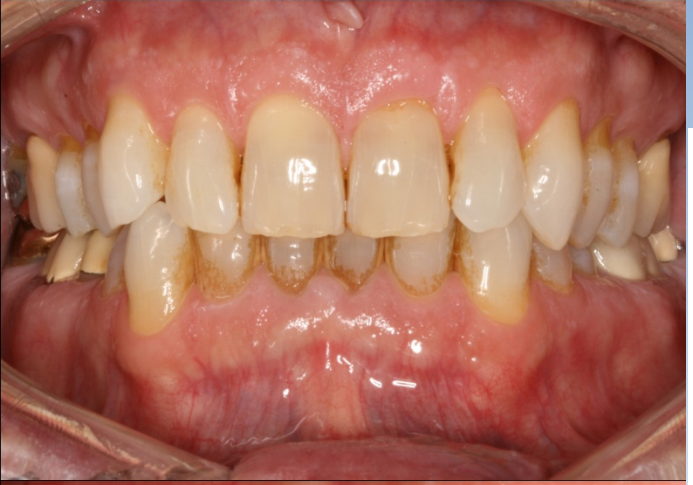
"Observed findings" provided by clinician

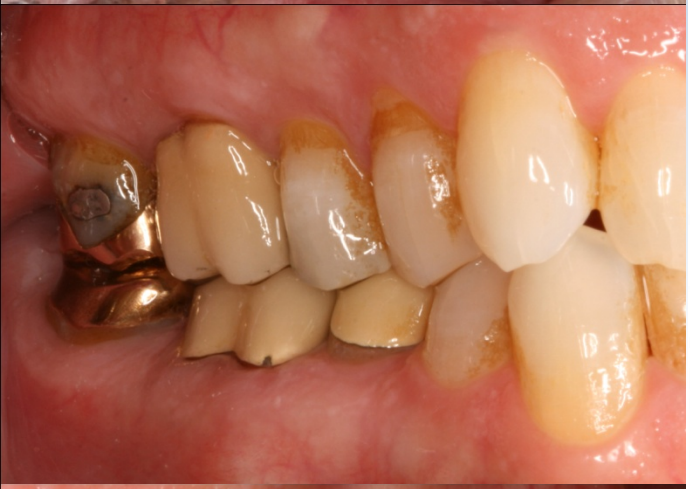
Antibiotic Allergies: None Reported

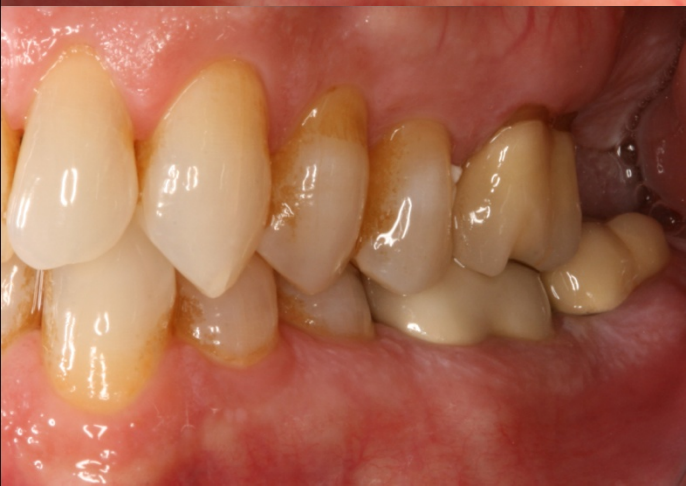
Tooth Numbers	2	3	5	10	14	15
Pocket Depths	6mm	8mm	9mm	5mm	7mm	7mm



Laboratory test results are objective quantifiable and reproducible







Bill

Birth Date: 12/4/1952

Medical History Date: 12/5/2011

Although dental personnel primarily treat the area in and around your mouth, your mouth is a part of your entire body. Health problems that you may have, or medication that you may be taking, could have an important interrelationship with the dentistry you will receive. Thank you for answering the following questions.

Are you under a physician's care now? ○ Yes ○ No If yes, please explain: _____

Have you ever been hospitalized or had a major operation? ○ Yes ○ No If yes, please explain: _____

Have you ever had a serious head or neck injury? ○ Yes ○ No If yes, please explain: _____

Are you taking any medications, pills, or drugs? ○ Yes ○ No 1 baby aspirin/ daily

Do you take, or have you taken, Phen-Fen or Redux? ○ Yes ○ No

Are you on a special diet? ○ Yes ○ No

Do you use tobacco? ○ Yes ○ No

Do you use controlled substances? ○ Yes ○ No

Women: Are you

☐ Pregnant/Trying to get pregnant? ☐ Nursing?

☐ Taking oral contraceptives?

Are you allergic to any of the following?

☐ Aspirin ☐ Penicillin ☐ Codeine ☐ Local Anesthetics ☐ Acrylic ☐ Metal ☐ Latex

☐ Other If other, please explain: _____

Do you have, or have you had, any of the following?

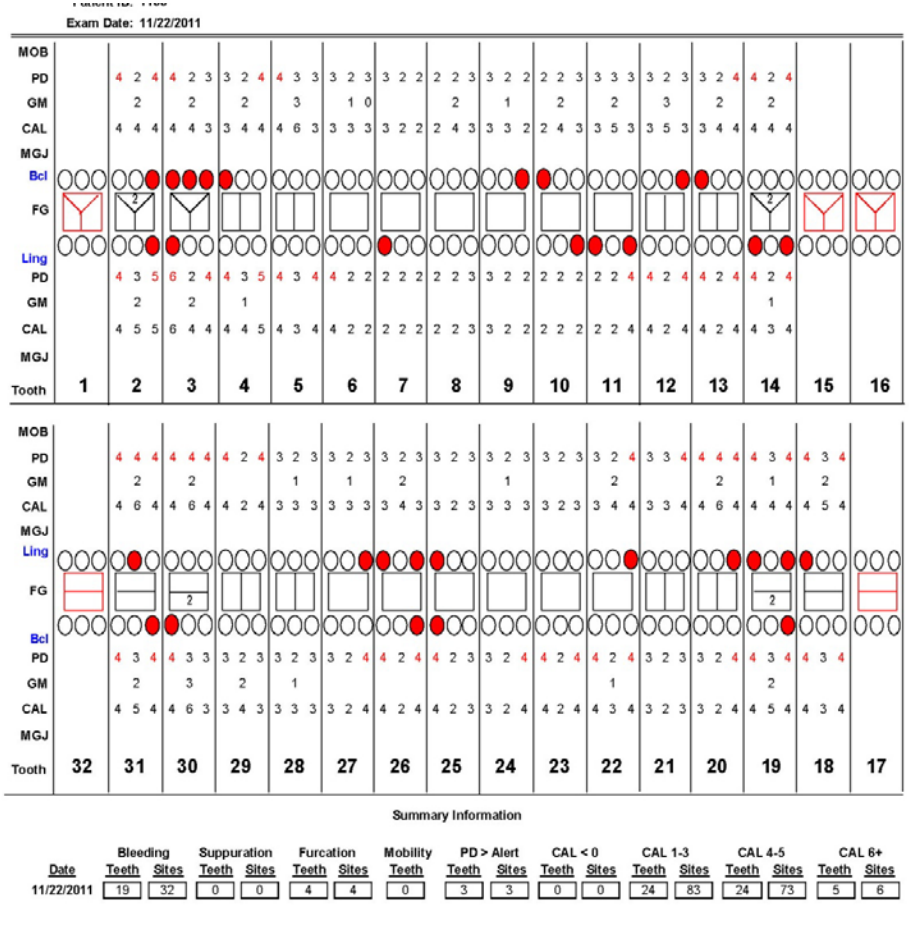
AIDS/HIV Positive	○ Yes ○ No	Cortisone Medicine	○ Yes ○ No	Hemophilia	○ Yes ○ No	Renal Dialysis	○ Yes ○ No
Alzheimer's Disease	○ Yes ○ No	Diabetes	○ Yes ○ No	Hepatitis A	○ Yes ○ No	Rheumatic Fever	○ Yes ○ No
Anaphylaxis	○ Yes ○ No	Drug Addiction	○ Yes ○ No	Hepatitis B or C	○ Yes ○ No	Rheumatism	○ Yes ○ No
Anemia	○ Yes ○ No	Easily Winded	○ Yes ○ No	Herpes	○ Yes ○ No	Scarlet Fever	○ Yes ○ No
Angina	○ Yes ○ No	Emphysema	○ Yes ○ No	High Blood Pressure	○ Yes ○ No	Shingles	○ Yes ○ No
Arthritis/Gout	○ Yes ○ No	Epilepsy or Seizures	○ Yes ○ No	Hives or Rash	○ Yes ○ No	Sickle Cell Disease	○ Yes ○ No
Artificial Heart Valve	○ Yes ○ No	Excessive Bleeding	○ Yes ○ No	Hypoglycemia	○ Yes ○ No	Sinus Trouble	○ Yes ○ No
Artificial Joint	○ Yes ○ No	Excessive Thirst	○ Yes ○ No	Irregular Heartbeat	○ Yes ○ No	Spina Bifida	○ Yes ○ No
Asthma	○ Yes ○ No	Fainting Spells/Dizziness	○ Yes ○ No	Kidney Problems	○ Yes ○ No	Stomach/Intestinal Disease	○ Yes ○ No
Blood Disease	○ Yes ○ No	Frequent Cough	○ Yes ○ No	Leukemia	○ Yes ○ No	Stroke	○ Yes ○ No
Blood Transfusion	○ Yes ○ No	Frequent Diarrhea	○ Yes ○ No	Liver Disease	○ Yes ○ No	Swelling of Limbs	○ Yes ○ No
Breathing Problem	○ Yes ○ No	Frequent Headaches	○ Yes ○ No	Low Blood Pressure	○ Yes ○ No	Thyroid Disease	○ Yes ○ No
Bruise Easily	○ Yes ○ No	Genital Herpes	○ Yes ○ No	Lung Disease	○ Yes ○ No	Tonsillitis	○ Yes ○ No
Cancer	○ Yes ○ No	Glaucoma	○ Yes ○ No	Mitral Valve Prolapse	○ Yes ○ No	Tuberculosis	○ Yes ○ No
Chemotherapy	○ Yes ○ No	Hay Fever	○ Yes ○ No	Pain in Jaw Joints	○ Yes ○ No	Tumors or Growths	○ Yes ○ No
Chest Pains	○ Yes ○ No	Heart Attack/Failure	○ Yes ○ No	Parathyroid Disease	○ Yes ○ No	Ulcers	○ Yes ○ No
Cold Sores/Fever Blisters	○ Yes ○ No	Heart Murmur	○ Yes ○ No	Psychiatric Care	○ Yes ○ No	Venereal Disease	○ Yes ○ No
Congenital Heart Disorder	○ Yes ○ No	Heart Pace Maker	○ Yes ○ No	Radiation Treatments	○ Yes ○ No	Yellow Jaundice	○ Yes ○ No
Convulsions	○ Yes ○ No	Heart Trouble/Disease	○ Yes ○ No	Recent Weight Loss	○ Yes ○ No		

Have you ever had any serious illness not listed above? ○ Yes ○ No If yes, please explain: _____

Only thing reported was laser treatment for Rosacea

Smoking one pack per day

Active Disease



Exam Date: 11/22/2011

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
MOB																
PD		4 2 4	4 2 3	3 2 4	4 3 3	3 2 3	3 2 2	2 2 3	3 2 2	2 2 3	3 3 3	3 2 3	3 2 4	4 2 4		
GM		2	2	2	3	1 0		2	1	2	2	3	2	2		
CAL		4 4 4	4 4 3	3 4 4	4 6 3	3 3 3	3 2 2	2 4 3	3 3 2	2 4 3	3 5 3	3 5 3	3 4 4	4 4 4		
MGJ																
Bcl																
FG		2												2		
Ling																
PD		4 3 5	6 2 4	4 3 5	4 3 4	4 2 2	2 2 2	2 2 3	3 2 2	2 2 2	2 2 4	4 2 4	4 2 4	4 2 4		
GM		2	2	1										1		
CAL		4 5 5	6 4 4	4 4 5	4 3 4	4 2 2	2 2 2	2 2 3	3 2 2	2 2 2	2 2 4	4 2 4	4 2 4	4 3 4		
MGJ																
Tooth	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16

	32	31	30	29	28	27	26	25	24	23	22	21	20	19	18	17
MOB																
PD		4 4 4	4 4 4	4 2 4	3 2 3	3 2 3	3 2 3	3 2 3	3 2 3	3 2 3	3 2 4	3 3 4	4 4 4	4 3 4	4 3 4	
GM		2	2		1	1	2		1		2		2	1	2	
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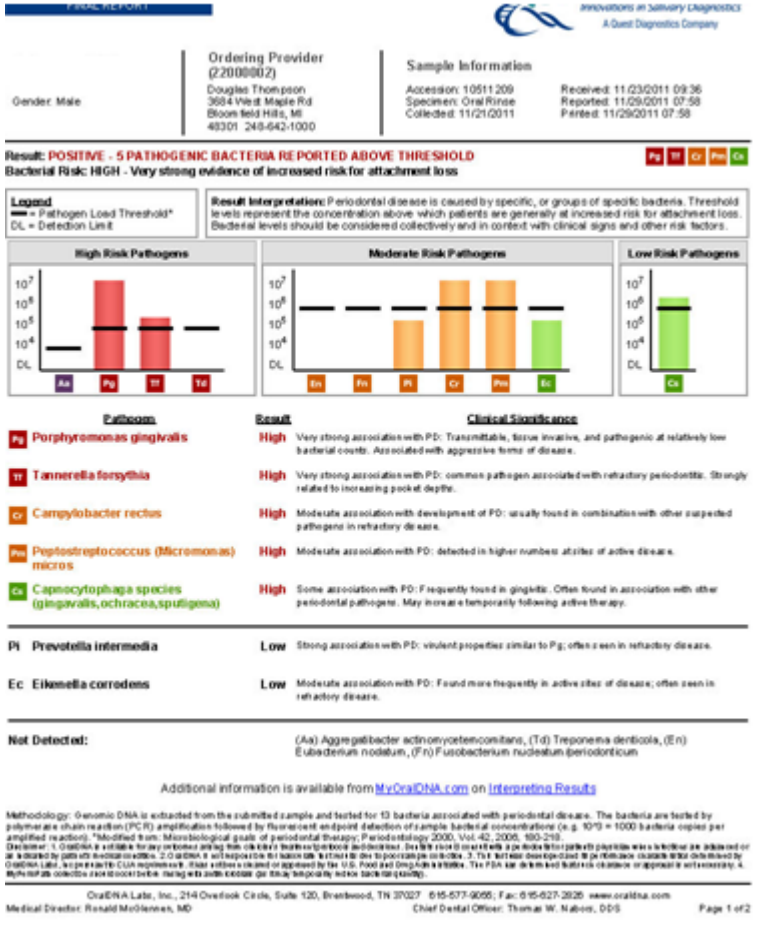
GM		2	3	2	1						1			2		
CAL		4 5 4	4 6 3	3 4 3	3 3 3	3 2 4	4 2 4	4 2 3	3 2 4	4 2 4	4 3 4	3 2 3	3 2 4	4 5 4	4 3 4	
MGJ																
Tooth	32	31	30	29	28	27	26	25	24	23	22	21	20	19	18	17

Summary Information

Date	Bleeding Teeth	Bleeding Sites	Suppuration Teeth	Suppuration Sites	Furcation Teeth	Furcation Sites	Mobility Teeth	PD > Alert Teeth	PD > Alert Sites	CAL < 0 Teeth	CAL < 0 Sites	CAL 1-3 Teeth	CAL 1-3 Sites	CAL 4-5 Teeth	CAL 4-5 Sites	CAL 6+ Teeth	CAL 6+ Sites
11/22/2011	19	32	0	0	4	4	0	3	3	0	0	24	83	24	73	5	6



Innovations in Salivary Diagnostics
A Quest Diagnostics Company

Ordering Provider (2200002)
Douglas Thompson
3684 West Maple Rd
Bloomfield Hills, MI
48301 248-642-1000

Gender: Male

Sample Information
Accession: 10511209
Specimen: Oral Rinse
Collected: 11/21/2011
Received: 11/23/2011 09:36
Reported: 11/29/2011 07:58
Printed: 11/29/2011 07:58

Result: POSITIVE - 5 PATHOGENIC BACTERIA REPORTED ABOVE THRESHOLD
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.

High Risk Pathogens | Moderate Risk Pathogens | Low Risk Pathogens

Pathogen	Result	Clinical Significance
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.
Tf Tannerella forsythia	High	Very strong association with PD: common pathogen associated with refractory periodontitis. Strongly related to increasing pocket depths.
Cr Campylobacter rectus	High	Moderate association with development of PD: usually found in combination with other suspected pathogens in refractory disease.
Pm Peptostreptococcus (Micromonas) micros	High	Moderate association with PD: detected in higher numbers at sites of active 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.
Pi Prevotella intermedia	Low	Strong association with PD: virulent properties similar to Pg; often seen in refractory disease.
Ec Eikenella corrodens	Low	Moderate association with PD: Found more frequently in active sites of disease; often seen in refractory disease.

Not Detected: (Aa) Aggregatibacter actinomycetemcomitans, (Td) Treponema denticola, (En) Eubacterium nodatum, (Fn) Fusobacterium nucleatum periodonticum

Additional information is available from MyOralDNA.com on Interpreting Results

Methodology: Genomic DNA is extracted from the submitted sample and tested for 13 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.

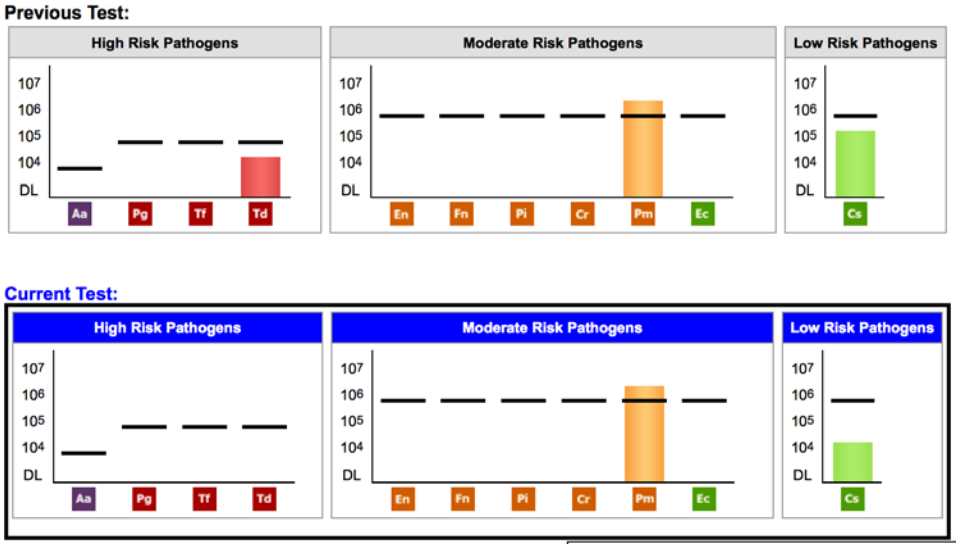
OralDNA Labs, Inc., 214 Overlook Circle, Suite 120, Brentwood, TN 37027 615-577-9066; Fax: 615-627-2926 www.oraldna.com
Medical Director: Ronald McGlennen, MD Chief Dental Officer: Thomas W. Nabors, DDS Page 1 of 2

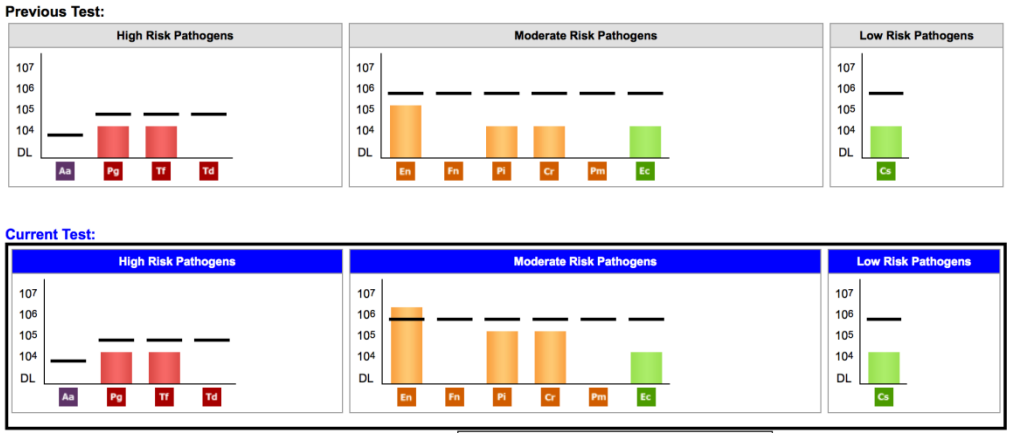
What Does the MyPerioPath® Test Teach Us?

- The collected clinical information is highly valuable in aggregate
- The bacterial “signature” is patient specific and lends to the decision of what therapy to apply
- Before and after results lend insight into the effectiveness of therapy

The Bacterial Pathogen Profile is a “Signature” of Your Patient’s Oral Health

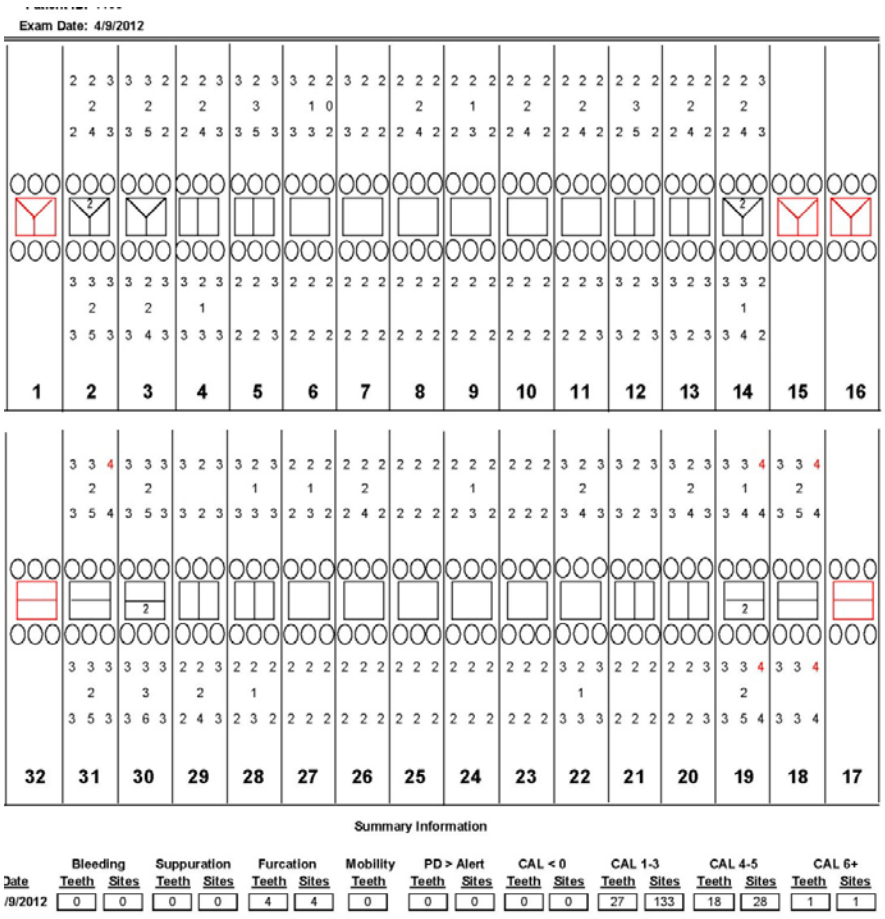
- 37 yr. old female with signs of moderate inflammation
- Prescribed a 8 day course of antibiotics
- Follow-up test suggests:
  - Pm and Cs refractory to treatment





- 42 yr. old male seen twice, separated by 6 months
- Encouraged to perform home care
- No other professional therapy between visits

Bill Treated to Stable Disease



Exam Date: 4/9/2012

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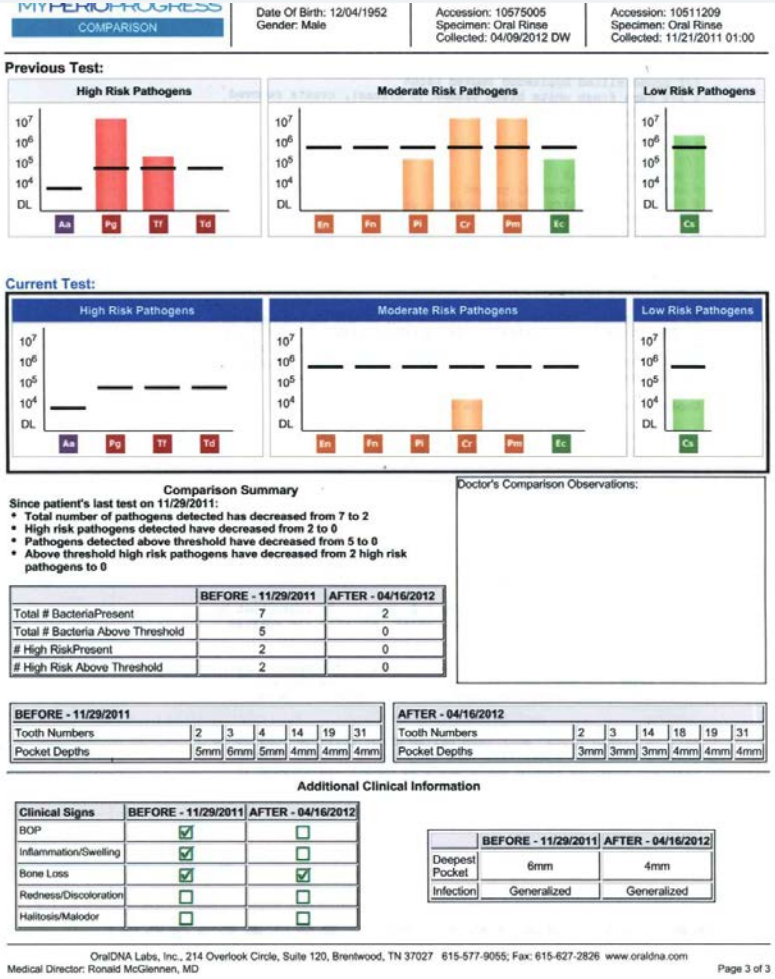
Summary Information

Date	Bleeding Teeth	Bleeding Sites	Suppuration Teeth	Suppuration Sites	Furcation Teeth	Furcation Sites	Mobility Teeth	PD > Alert Teeth	PD > Alert Sites	CAL < 0 Teeth	CAL < 0 Sites	CAL 1-3 Teeth	CAL 1-3 Sites	CAL 4-5 Teeth	CAL 4-5 Sites	CAL 6+ Teeth	CAL 6+ Sites
/9/2012	0	0	0	0	4	4	0	0	0	0	0	27	133	18	28	1	1



MYPERIOPROGRESS

COMPARISON

Date Of Birth: 12/04/1952
Gender: Male

Accession: 10575005
Specimen: Oral Rinse
Collected: 04/09/2012 DW

Accession: 10511209
Specimen: Oral Rinse
Collected: 11/21/2011 01:00

Previous Test:

High Risk Pathogens | Moderate Risk Pathogens | Low Risk Pathogens

10^7 10^6 10^5 10^4 DL

Aa Pg Tf Td | En Fn Pi Cr Pm Ec | Cs

Current Test:

High Risk Pathogens | Moderate Risk Pathogens | Low Risk Pathogens

10^7 10^6 10^5 10^4 DL

Aa Pg Tf Td | En Fn Pi Cr Pm Ec | Cs

Comparison Summary

Since patient's last test on 11/29/2011:

- Total number of pathogens detected has decreased from 7 to 2
- High risk pathogens detected have decreased from 2 to 0
- Pathogens detected above threshold have decreased from 5 to 0
- Above threshold high risk pathogens have decreased from 2 high risk pathogens to 0

Doctor's Comparison Observations:

	BEFORE - 11/29/2011	AFTER - 04/16/2012
Total # BacteriaPresent	7	2
Total # Bacteria Above Threshold	5	0
# High RiskPresent	2	0
# High Risk Above Threshold	2	0

BEFORE - 11/29/2011						
Tooth Numbers	2	3	4	14	19	31
Pocket Depths	5mm	6mm	5mm	4mm	4mm	4mm

AFTER - 04/16/2012						
Tooth Numbers	2	3	14	18	19	31
Pocket Depths	3mm	3mm	3mm	4mm	4mm	4mm

Additional Clinical Information

Clinical Signs	BEFORE - 11/29/2011	AFTER - 04/16/2012
BOP	☑	☐
Inflammation/Swelling	☑	☐
Bone Loss	☑	☑
Redness/Discoloration	☐	☐
Halitosis/Malodor	☐	☐

	BEFORE - 11/29/2011	AFTER - 04/16/2012
Deepest Pocket	6mm	4mm
Infection	Generalized	Generalized

OralDNA Labs, Inc., 214 Overlook Circle, Suite 120, Brentwood, TN 37027 615-577-9055; Fax: 615-627-2826 www.oraldna.com
Medical Director: Ronald McGlennen, MD

Page 3 of 3

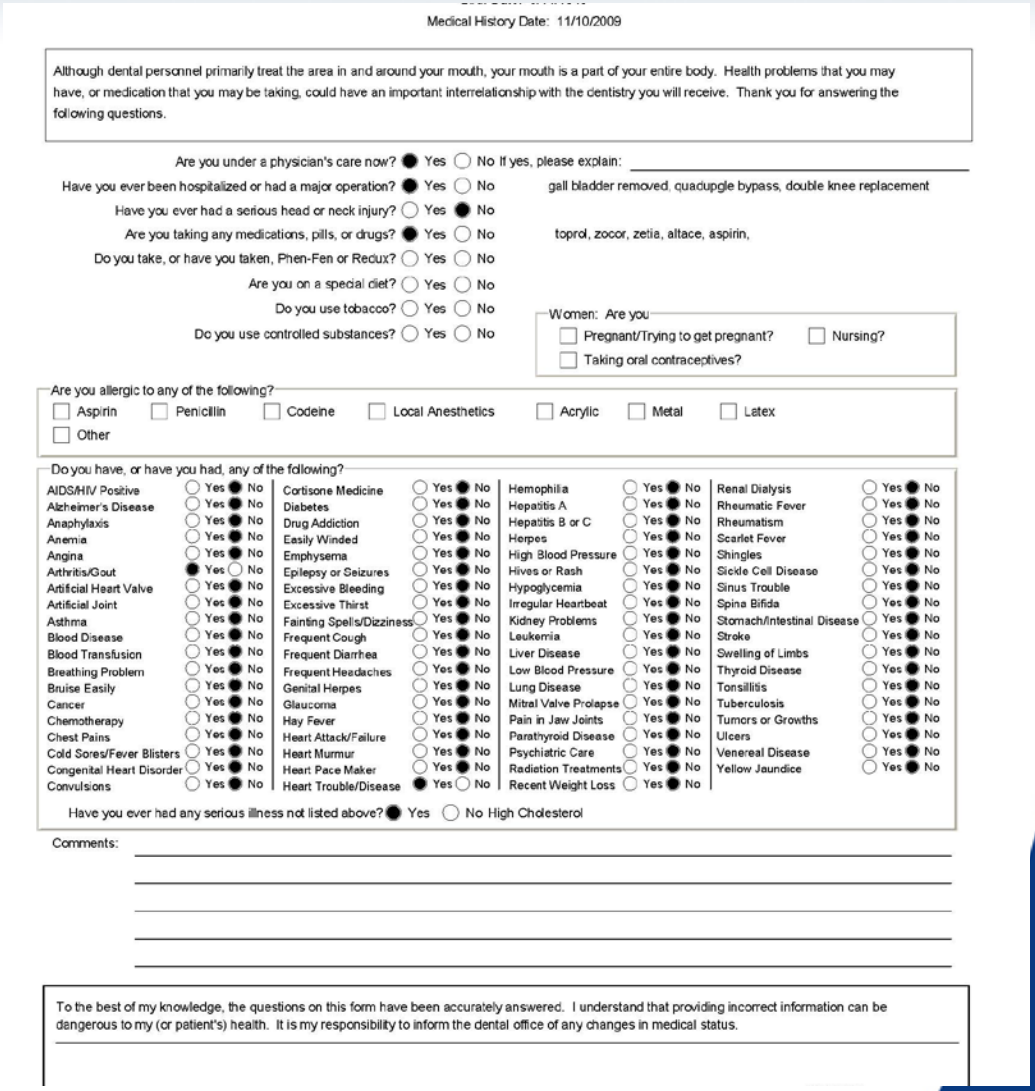
Post Treatment

Case Study

David is a 66 year old male

Medical History

- History of quadruple coronary artery bypass-2008
- History of double knee replacement-2007
- Medical conditions:
  - Hypercholesterolemia
  - Osteoarthritis
- Current Medications:
  - Toprol and Alstace
  - Side effect of xerostomia



Medical History Date: 11/10/2009

Although dental personnel primarily treat the area in and around your mouth, your mouth is a part of your entire body. Health problems that you may have, or medication that you may be taking, could have an important interrelationship with the dentistry you will receive. Thank you for answering the following questions.

Are you under a physician's care now? ● Yes ○ No If yes, please explain: _____

Have you ever been hospitalized or had a major operation? ● Yes ○ No — gall bladder removed, quadupgle bypass, double knee replacement

Have you ever had a serious head or neck injury? ○ Yes ● No

Are you taking any medications, pills, or drugs? ● Yes ○ No — toprol, zocor, zetia, altace, aspirin,

Do you take, or have you taken, Phen-Fen or Redux? ○ Yes ○ No

Are you on a special diet? ○ Yes ○ No

Do you use tobacco? ○ Yes ○ No

Do you use controlled substances? ○ Yes ○ No

Women: Are you
☐ Pregnant/Trying to get pregnant? ☐ Nursing?
☐ Taking oral contraceptives?

Are you allergic to any of the following?
☐ Aspirin ☐ Penicillin ☐ Codeine ☐ Local Anesthetics ☐ Acrylic ☐ Metal ☐ Latex
☐ Other

Do you have, or have you had, any of the following?

AIDS/HIV Positive	○ Yes ● No	Cortisone Medicine	○ Yes ● No	Hemophilia	○ Yes ● No	Renal Dialysis	○ Yes ● No
Alzheimer's Disease	○ Yes ● No	Diabetes	○ Yes ● No	Hepatitis A	○ Yes ● No	Rheumatic Fever	○ Yes ● No
Anaphylaxis	○ Yes ● No	Drug Addiction	○ Yes ● No	Hepatitis B or C	○ Yes ● No	Rheumatism	○ Yes ● No
Anemia	○ Yes ● No	Easily Winded	○ Yes ● No	Herpes	○ Yes ● No	Scarlet Fever	○ Yes ● No
Angina	○ Yes ● No	Emphysema	○ Yes ● No	High Blood Pressure	○ Yes ● No	Shingles	○ Yes ● No
Arthritis/Gout	● Yes ○ No	Epilepsy or Seizures	○ Yes ● No	Hives or Rash	○ Yes ● No	Sickle Cell Disease	○ Yes ● No
Artificial Heart Valve	○ Yes ● No	Excessive Bleeding	○ Yes ● No	Hypoglycemia	○ Yes ● No	Sinus Trouble	○ Yes ● No
Artificial Joint	○ Yes ● No	Excessive Thirst	○ Yes ● No	Irregular Heartbeat	○ Yes ● No	Spina Bifida	○ Yes ● No
Asthma	○ Yes ● No	Fainting Spells/Dizziness	○ Yes ● No	Kidney Problems	○ Yes ● No	Stomach/Intestinal Disease	○ Yes ● No
Blood Disease	○ Yes ● No	Frequent Cough	○ Yes ● No	Leukemia	○ Yes ● No	Stroke	○ Yes ● No
Blood Transfusion	○ Yes ● No	Frequent Diarrhea	○ Yes ● No	Liver Disease	○ Yes ● No	Swelling of Limbs	○ Yes ● No
Breathing Problem	○ Yes ● No	Frequent Headaches	○ Yes ● No	Low Blood Pressure	○ Yes ● No	Thyroid Disease	○ Yes ● No
Bruise Easily	○ Yes ● No	Genital Herpes	○ Yes ● No	Lung Disease	○ Yes ● No	Tonsillitis	○ Yes ● No
Cancer	○ Yes ● No	Glaucoma	○ Yes ● No	Mitral Valve Prolapse	○ Yes ● No	Tuberculosis	○ Yes ● No
Chemotherapy	○ Yes ● No	Hay Fever	○ Yes ● No	Pain in Jaw Joints	○ Yes ● No	Tumors or Growths	○ Yes ● No
Chest Pains	○ Yes ● No	Heart Attack/Failure	○ Yes ● No	Parathyroid Disease	○ Yes ● No	Ulcers	○ Yes ● No
Cold Sores/Fever Blisters	○ Yes ● No	Heart Murmur	○ Yes ● No	Psychiatric Care	○ Yes ● No	Venereal Disease	○ Yes ● No
Congenital Heart Disorder	○ Yes ● No	Heart Pace Maker	○ Yes ● No	Radiation Treatments	○ Yes ● No	Yellow Jaundice	○ Yes ● No
Convulsions	○ Yes ● No	Heart Trouble/Disease	● Yes ○ No	Recent Weight Loss	○ Yes ● No		

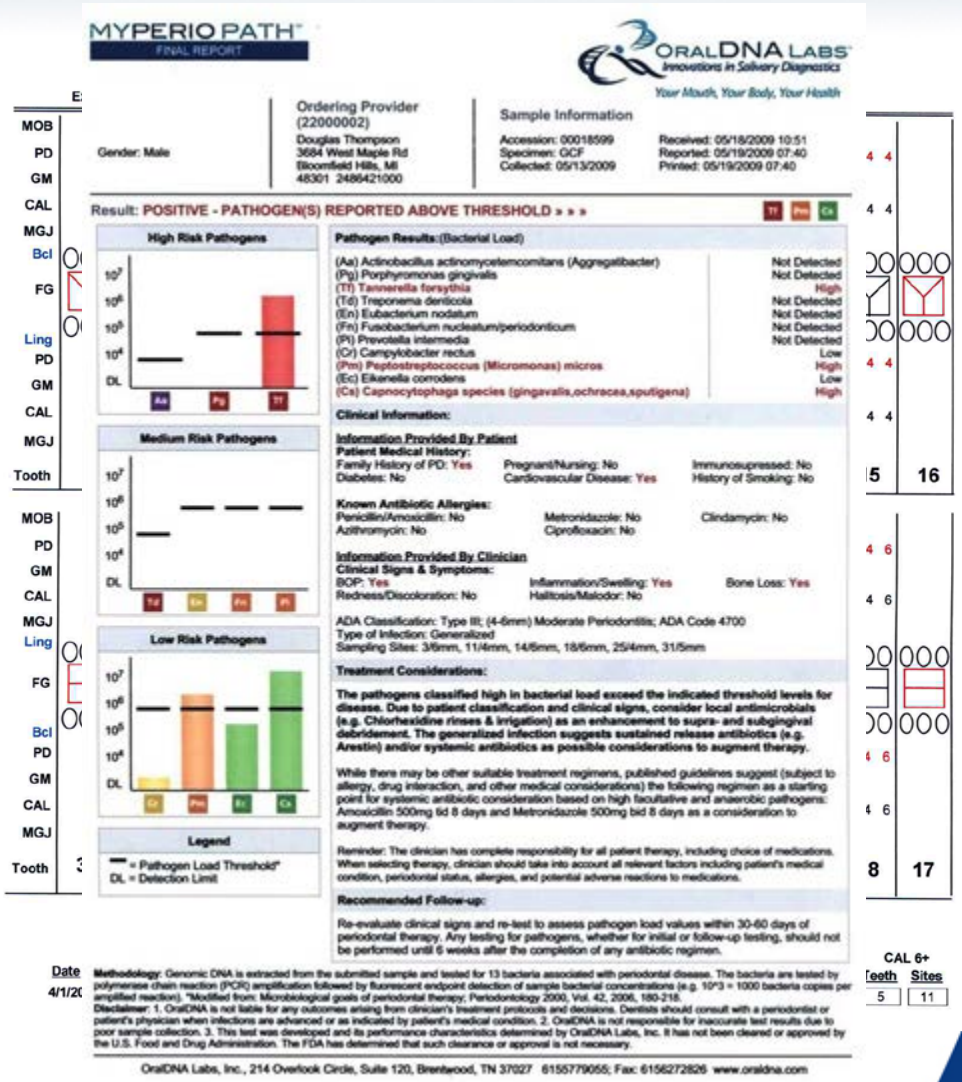
Have you ever had any serious illness not listed above? ● Yes ○ No High Cholesterol

Comments: _____

To the best of my knowledge, the questions on this form have been accurately answered. I understand that providing incorrect information can be dangerous to my (or patient's) health. It is my responsibility to inform the dental office of any changes in medical status.

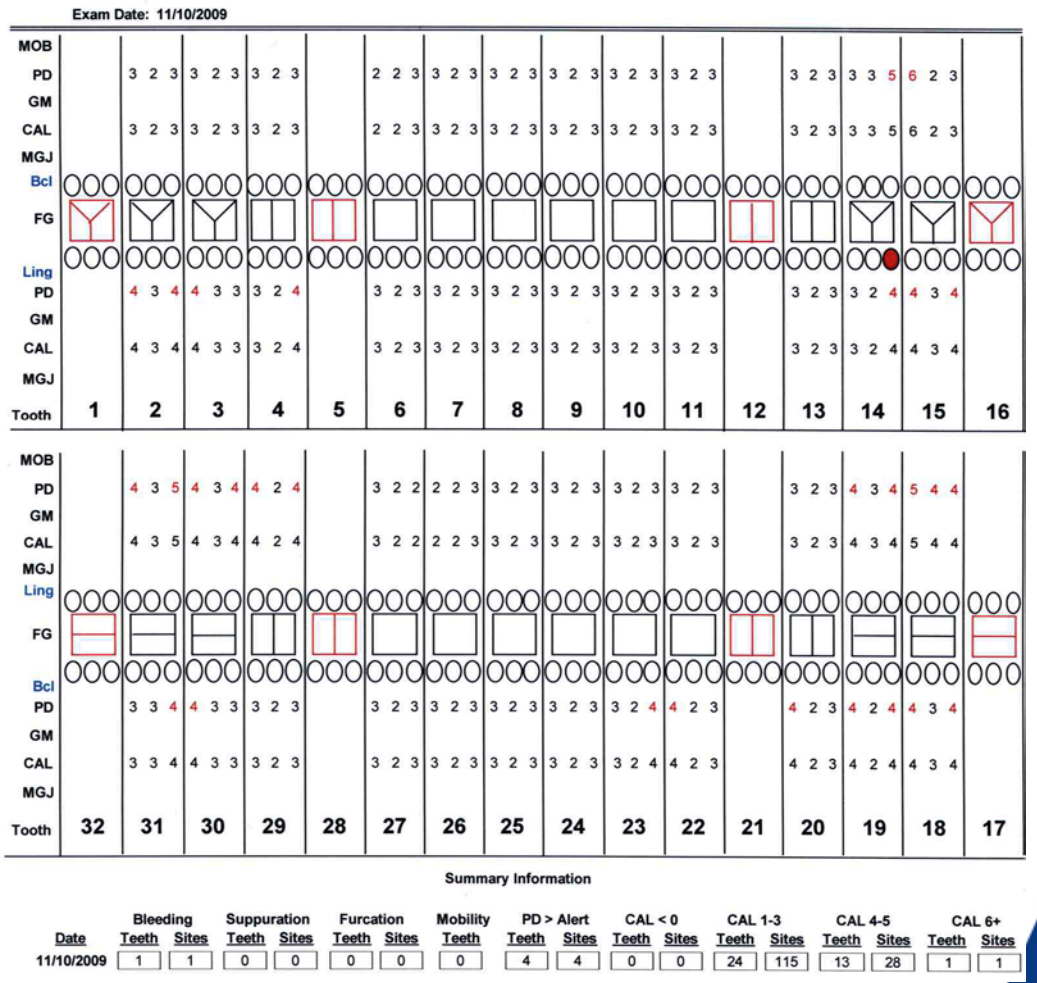
Heavy Bleeding Noted

- In April 2009, heavy bleeding noted
- AAP IV- active disease
- Discussion with patient the need for testing to assist with diagnosis
- Consider the risk modifiers and history of prior bone loss


MYPERIO PATH
FINAL REPORT
ORALDNA LABS
Innovations in Salivary Diagnostics
Your Mouth, Your Body, Your Health
Ordering Provider (22000002)
Douglas Thompson
3684 West Maple Rd
Bloomfield Hills, MI
48301 2486421000
Sample Information
Accession: 00018599
Specimen: GCF
Collected: 05/13/2009
Received: 05/18/2009 10:51
Reported: 05/19/2009 07:40
Printed: 05/19/2009 07:40
Gender: Male
Result: POSITIVE - PATHOGEN(S) REPORTED ABOVE THRESHOLD » » »
High Risk Pathogens
Medium Risk Pathogens
Low Risk Pathogens
Legend
= Pathogen Load Threshold*
DL = Detection Limit
Pathogen Results:(Bacterial Load)
(Aa) Actinobacillus actinomycetemcomitans (Aggregatibacter) Not Detected
(Pg) Porphyromonas gingivalis Not Detected
(Tf) Tannerella forsythia High
(Td) Treponema denticola Not Detected
(En) Eubacterium nodatum Not Detected
(Fn) Fusobacterium nucleatum/periodonticum Not Detected
(Pi) Prevotella intermedia Not Detected
(Cr) Campylobacter rectus Low
(Pm) Peptostreptococcus (Micromonas) micros High
(Ec) Eikenella corrodens Low
(Ca) Capnocytophaga species (gingavalis,ochracea,sputigena) High
Clinical Information:
Information Provided By Patient
Patient Medical History:
Family History of PD: Yes
Diabetes: No
Pregnant/Nursing: No
Cardiovascular Disease: Yes
Immunosupressed: No
History of Smoking: No
Known Antibiotic Allergies:
Penicillin/Amoxicillin: No
Azithromycin: No
Metronidazole: No
Ciprofloxacin: No
Clindamycin: No
Information Provided By Clinician
Clinical Signs & Symptoms:
BOP: Yes
Redness/Discoloration: No
Inflammation/Swelling: Yes
Halitosis/Malodor: No
Bone Loss: Yes
ADA Classification: Type III; (4-6mm) Moderate Periodontitis; ADA Code 4700
Type of Infection: Generalized
Sampling Sites: 3/6mm, 11/4mm, 14/6mm, 18/6mm, 25/4mm, 31/5mm
Treatment Considerations:
The pathogens classified high in bacterial load exceed the indicated threshold levels for disease. Due to patient classification and clinical signs, consider local antimicrobials (e.g. Chlorhexidine rinses & irrigation) as an enhancement to supra- and subgingival debridement. The generalized infection suggests sustained release antibiotics (e.g. Arestin) and/or systemic antibiotics as possible considerations to augment therapy.
While there may be other suitable treatment regimens, published guidelines suggest (subject to allergy, drug interaction, and other medical considerations) the following regimen as a starting point for systemic antibiotic consideration based on high facultative and anaerobic pathogens: Amoxicillin 500mg tid 8 days and Metronidazole 500mg bid 8 days as a consideration to augment therapy.
Reminder: The clinician has complete responsibility for all patient therapy, including choice of medications. When selecting therapy, clinician should take into account all relevant factors including patient's medical condition, periodontal status, allergies, and potential adverse reactions to medications.
Recommended Follow-up:
Re-evaluate clinical signs and re-test to assess pathogen load values within 30-60 days of periodontal therapy. Any testing for pathogens, whether for initial or follow-up testing, should not be performed until 6 weeks after the completion of any antibiotic regimen.
Methodology: Genomic DNA is extracted from the submitted sample and tested for 13 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, Inc. 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.
OralDNA Labs, Inc., 214 Overlook Circle, Suite 120, Brentwood, TN 37027 6155779055; Fax: 6156272826 www.oraldna.com
MOB PD GM CAL MGJ Bcl FG Ling PD GM CAL MGJ Tooth
MOB PD GM CAL MGJ Ling FG Bcl PD GM CAL MGJ Tooth
4 4 4 4 4 4 5 16 4 6 4 6 4 6 8 17
Date 4/1/2
CAL 6+ Teeth 5 Sites 11

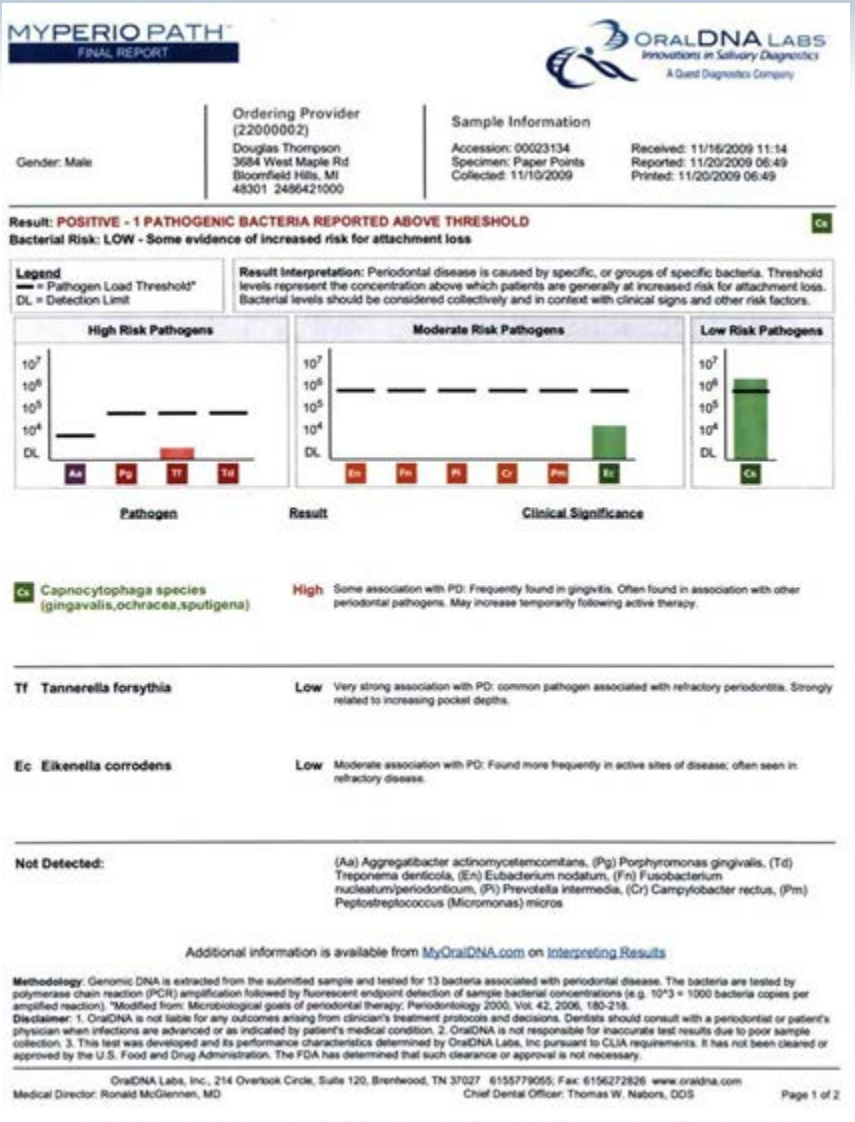
Clinical Course

- Pre-treatment clinical chart
- Post-treatment clinical chart
- Fantastic reduction in the number of bleeding sites and pocket depth



Treatment Results

- Risk Modifier Summary
- Radiographic bone loss
- Earlier site specific care
- Family history of gum disease (mom)
- Heart disease
- Borderline diabetic
- Stress (three recent surgeries)
- Double knee replacement



MYPERIO PATH
FINAL REPORT

ORALDNA LABS
Innovations in Salivary Diagnostics
A Quest Diagnostics Company

Gender: Male

Ordering Provider (22000002)
Douglas Thompson
3684 West Maple Rd
Bloomfield Hills, MI
48301 2486421000

Sample Information
Accession: 00023134
Specimen: Paper Points
Collected: 11/10/2009
Received: 11/16/2009 11:14
Reported: 11/20/2009 06:49
Printed: 11/20/2009 06:49

Result: POSITIVE - 1 PATHOGENIC BACTERIA REPORTED ABOVE THRESHOLD
Bacterial Risk: LOW - Some 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.

High Risk Pathogens | Moderate Risk Pathogens | Low Risk Pathogens

10^7 10^6 10^5 10^4 DL

Aa Pg Tf Td | En Fn Pi Cr Pm Ec | Cs

Pathogen	Result	Clinical Significance
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.
Tf Tannerella forsythia	Low	Very strong association with PD: common pathogen associated with refractory periodontitis. Strongly related to increasing pocket depths.
Ec Eikenella corrodens	Low	Moderate association with PD: Found more frequently in active sites of disease; often seen in refractory disease.

Not Detected: (Aa) Aggregatibacter actinomycetemcomitans, (Pg) Porphyromonas gingivalis, (Td) Treponema denticola, (En) Eubacterium nodatum, (Fn) Fusobacterium nucleatum/periodonticum, (Pi) Prevotella intermedia, (Cr) Campylobacter rectus, (Pm) Peptostreptococcus (Micromonas) micros

Additional information is available from MyOralDNA.com on Interpreting Results

Methodology: Genomic DNA is extracted from the submitted sample and tested for 13 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.
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Medical Director: Ronald McGlennen, MD Chief Dental Officer: Thomas W. Nabors, DDS Page 1 of 2

Summary

Date	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
B 11/10/2009		3 2 3	3 2 3	3 2 3		2 2 3	3 2 3	3 2 3	3 2 3	3 2 3	3 2 3		3 2 3	3 3 5	6 2 3	
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11/12/2007		2 2 4	6 2 3	3 2 3		3 2 2	2 2 2	2 2 3	3 2 3	3 2 3	3 2 3		3 2 3	3 2 5	4 2 3	
L 11/10/2009		4 3 4	4 3 3	3 2 4		3 2 3	3 2 3	3 2 3	3 2 3	3 2 3	3 2 3		3 2 3	3 2 4	4 3 4	
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11/12/2007		5 3 5	5 3 5	4 4 2		4 4 2	3 3 2	3 3 2	3 3 2	3 3 2	3 3 2		3 3 2	4 3 5	4 3 3	

Date	32	31	30	29	28	27	26	25	24	23	22	21	20	19	18	17
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4/1/2009		4 4 5	5 4 4	4 4 4		3 3 3	3 3 3	3 2 3	3 2 3	3 3 4	4 2 4		4 4 4	5 4 6	6 4 6	
11/12/2007		4 3 5	5 3 5	4 3 3		3 2 3	3 3 3	3 2 3	3 2 3	3 2 3	3 2 3		3 3 3	3 3 4	4 4 3	
B 11/10/2009		3 3 4	4 3 3	3 2 3		3 2 3	3 2 3	3 2 3	3 2 3	3 2 4	4 2 3		4 2 3	4 2 4	4 3 4	
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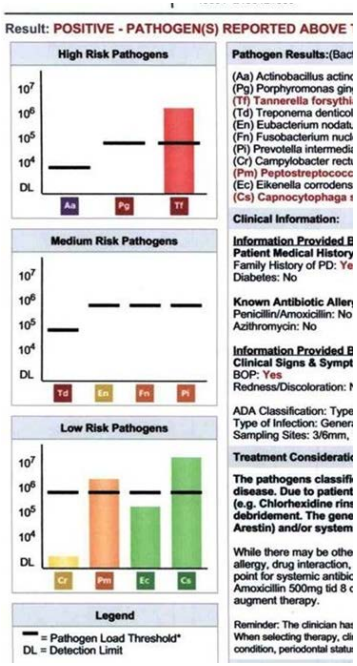
11/12/2007		3 3 4	4 3 3	3 2 3		3 2 3	3 2 3	3 2 3	3 3 3	3 3 4	4 3 4		4 3 4	3 2 4	5 2 3	

Summary Data Comparison

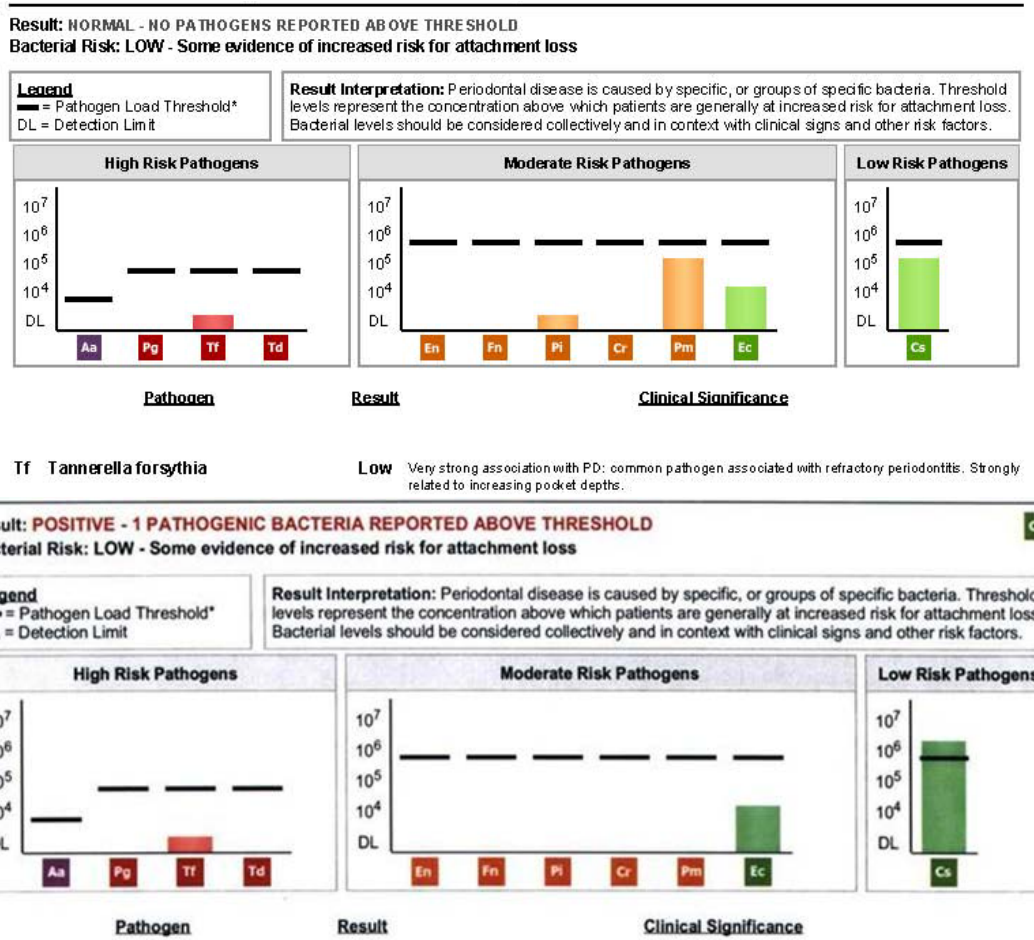
Date	Bleeding Teeth	Bleeding Sites	Suppuration Teeth	Suppuration Sites	Furcation Teeth	Furcation Sites	Mobility Teeth	PD > Alert Teeth	PD > Alert Sites	CAL < 0 Teeth	CAL < 0 Sites	CAL 1-3 Teeth	CAL 1-3 Sites	CAL 4-5 Teeth	CAL 4-5 Sites	CAL 6+ Teeth	CAL 6+ Sites
11/10/2009	1	1	0	0	0	0	0	4	4	0	0	24	115	13	28	1	1
4/1/2009	23	60	0	0	0	0	0	9	25	0	0	19	68	20	65	5	11
11/12/2007	23	44	0	0	0	0	0	6	11	0	0	24	112	14	31	1	1

Two Years Post Treatment

- Patient chart- 2 years post treatment

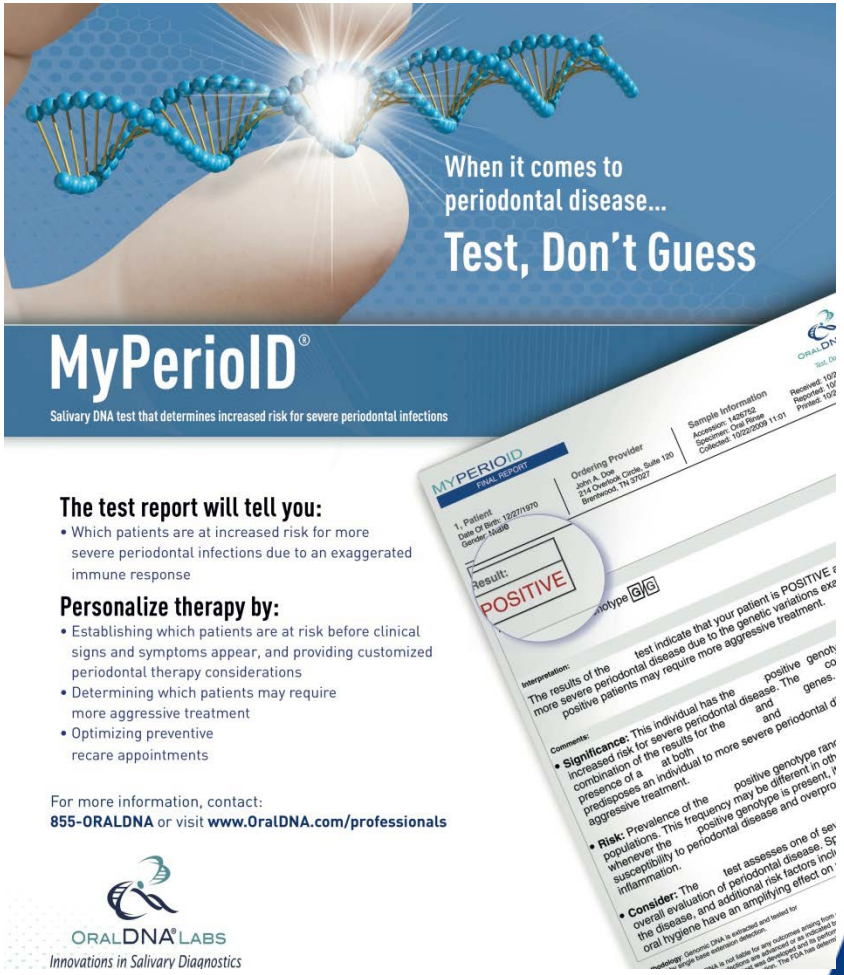


Initial MPP Result



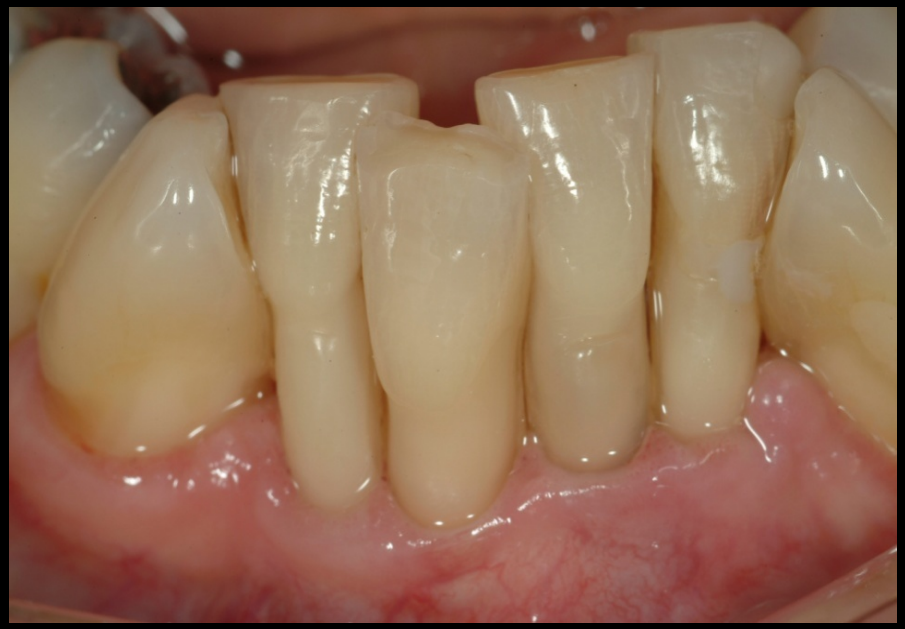
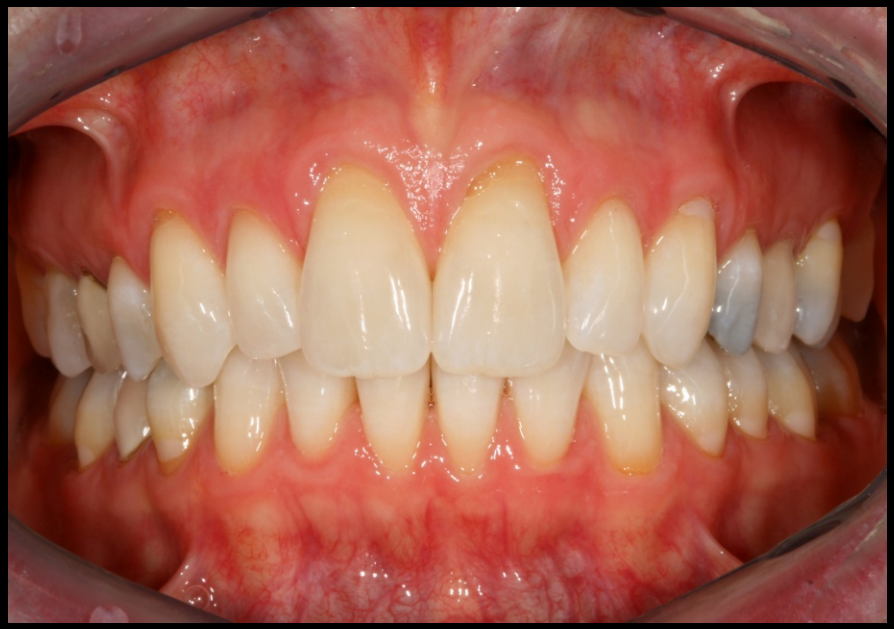
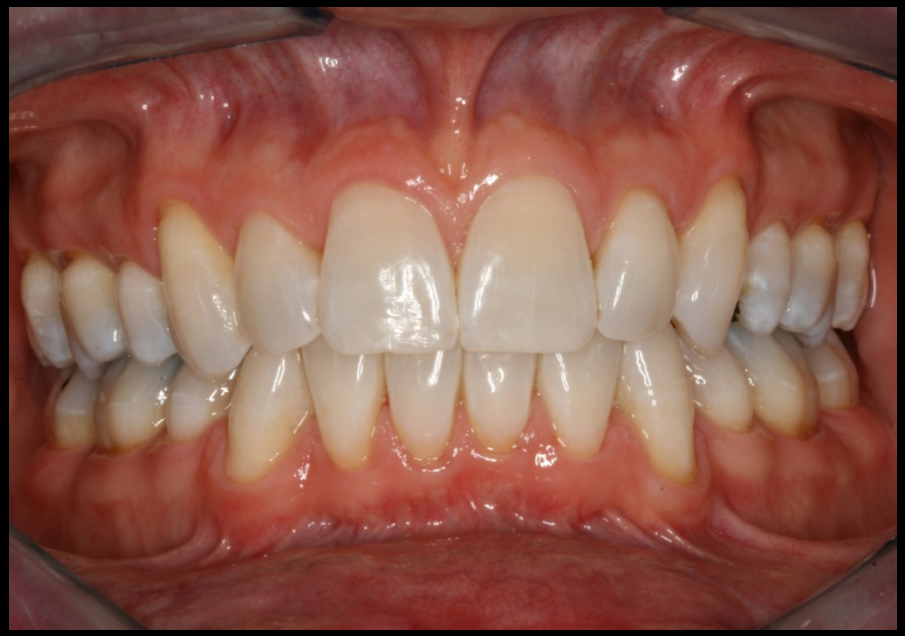
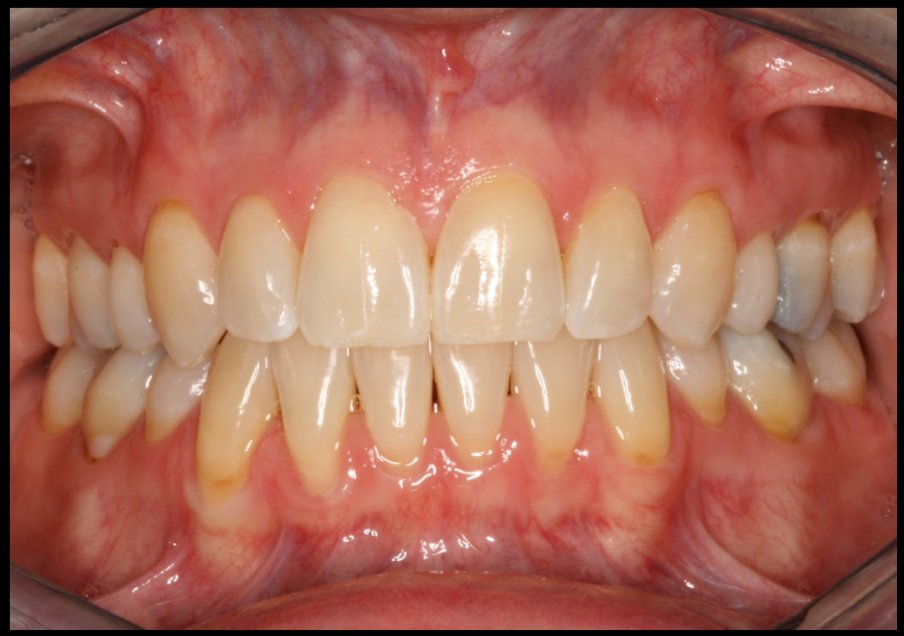
Beyond Bacteria- Your Inherited Risk of Periodontitis

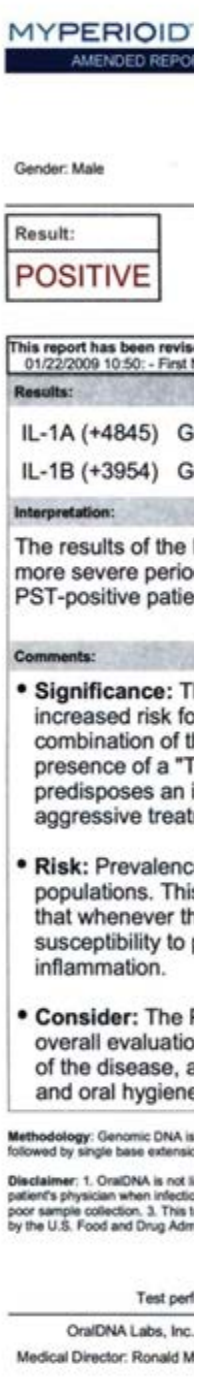
- Pathogenic bacteria are only part of the story
- A person inherent genetic risk is a key component of one's susceptibility to develop periodontitis
- Genetic markers are predictive of
  - The onset of clinical disease
  - The rate of progression
  - The likely prognosis



The Genetic Risk of Periodontitis

- Three conditions to consider:
  - Heritable forms of periodontitis-*rare*
  - Genetic susceptibility to periodontitis-*very common*
  - Other diseases masking as periodontitis- v*ariable*
- More than 30% of test requests provide clinical report of “family history of PD”
  - Construction of a classic family pedigree does not support the majority of these cases as being heritable
  - Yet, what is the magnitude of that concern that PD runs in families?


MYPERIOID
AMENDED REPO

Gender: Male

Result:
POSITIVE

This report has been revis
01/22/2009 10:50: - First

Results:

IL-1A (+4845) G
IL-1B (+3954) G

Interpretation:

The results of the
more severe perio
PST-positive patie

Comments:

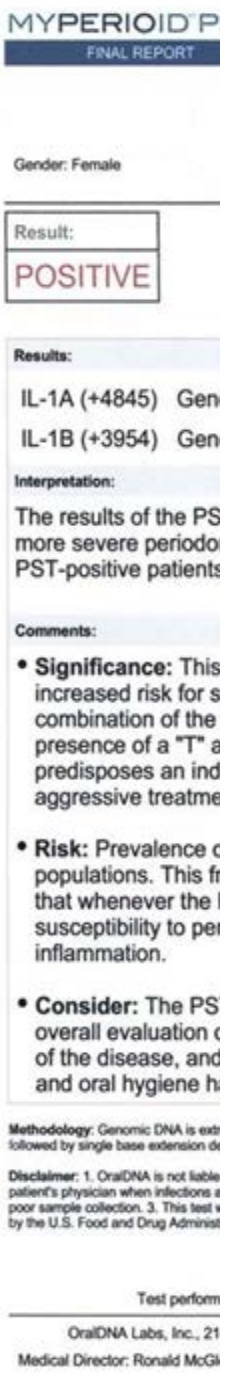
- **Significance:** T
increased risk fo
combination of t
presence of a "T
predisposes an i
aggressive treat
- **Risk:** Prevalenc
populations. This
that whenever th
susceptibility to
inflammation.
- **Consider:** The
overall evaluatio
of the disease, a
and oral hygiene

Methodology: Genomic DNA is
followed by single base extensi

Disclaimer: 1. OralDNA is not
patient's physician when infectio
poor sample collection. 3. This t
by the U.S. Food and Drug Adm

Test perf

OralDNA Labs, Inc.
Medical Director: Ronald M


MYPERIOID P
FINAL REPORT

Gender: Female

Result:
POSITIVE

Results:

IL-1A (+4845) Gen
IL-1B (+3954) Gen

Interpretation:

The results of the PS
more severe periodo
PST-positive patients

Comments:

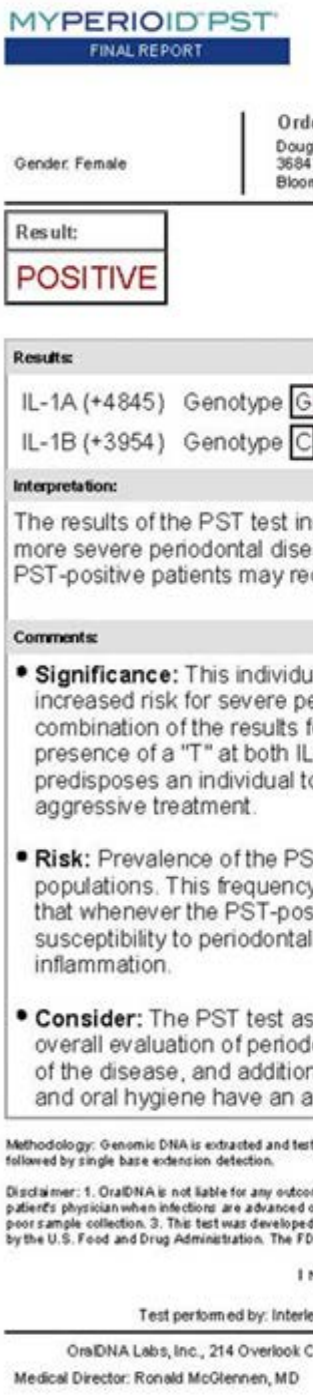
- **Significance:** This
increased risk for s
combination of the
presence of a "T" a
predisposes an ind
aggressive treatme
- **Risk:** Prevalence o
populations. This fr
that whenever the
susceptibility to per
inflammation.
- **Consider:** The PS
overall evaluation o
of the disease, and
and oral hygiene h

Methodology: Genomic DNA is extr
followed by single base extension d

Disclaimer: 1. OralDNA is not liable
patient's physician when infections a
poor sample collection. 3. This test
by the U.S. Food and Drug Administ

Test perform

OralDNA Labs, Inc., 21
Medical Director: Ronald McGl


MYPERIOID PST
FINAL REPORT

Gender: Female

Ord
Doug
3684
Bloo

Result:
POSITIVE

Results:

IL-1A (+4845) Genotype G
IL-1B (+3954) Genotype C

Interpretation:

The results of the PST test in
more severe periodontal dise
PST-positive patients may re

Comments:

- **Significance:** This individu
increased risk for severe pe
combination of the results f
presence of a "T" at both IL
predisposes an individual t
aggressive treatment.
- **Risk:** Prevalence of the PS
populations. This frequency
that whenever the PST-pos
susceptibility to periodontal
inflammation.
- **Consider:** The PST test as
overall evaluation of period
of the disease, and addition
and oral hygiene have an a

Methodology: Genomic DNA is extracted and test
followed by single base extension detection.

Disclaimer: 1. OralDNA is not liable for any outco
patient's physician when infections are advanced
poor sample collection. 3. This test was developed
by the U.S. Food and Drug Administration. The FD

Test performed by: Interle

OralDNA Labs, Inc., 214 Overlook C
Medical Director: Ronald McGlennen, MD

MYPERIOID® PST®
FINAL REPORT

ORALDNA LABS
Innovations in Salivary Diagnostics
A Quest Diagnostics Company

Gender: Female

Ordering Provider
Douglas Thompson
3684 West Maple Rd
Bloomfield Hills, MI
48301 248-642-1000

Sample Information
Accession: 10170406
Specimen: Oral Rinse
Collected: 10/21/2010
Received: 10/26/2010 10:28
Reported: 11/11/2010 13:59
Printed: 11/11/2010 13:59

Result:
POSITIVE

Results:

IL-1A (+4845) Genotype G/T
IL-1B (+3954) Genotype C/T

Interpretation:

The results of the PST test indicate that your patient is POSITIVE and has an increased risk for more severe periodontal disease due to the genetic variations examined in this test. PST-positive patients may require more aggressive treatment.

Comments:

- **Significance:** This individual has the "PST-positive" genotype and is therefore at a 3-7 fold increased risk for severe periodontal disease. The PST composite genotype is based on the combination of the results for the IL-1A and IL-1B genes. Any combination that includes the presence of a "T" at both IL-1A (+4845) and IL-1B (+3954) is defined as PST-positive and predisposes an individual to more severe periodontal disease which may require more aggressive treatment.
- **Risk:** Prevalence of the PST-positive genotype ranges from 30 to 40% in Caucasian populations. This frequency may be different in other ethnic groups. It is important to note that whenever the PST-positive genotype is present, it is associated with an increased susceptibility to periodontal disease and overproduction of IL-1, a cytokine that amplifies inflammation.
- **Consider:** The PST 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 disease, and additional risk factors including genetic susceptibility, smoking, diabetes, and oral hygiene have an amplifying effect on periodontal disease progression.

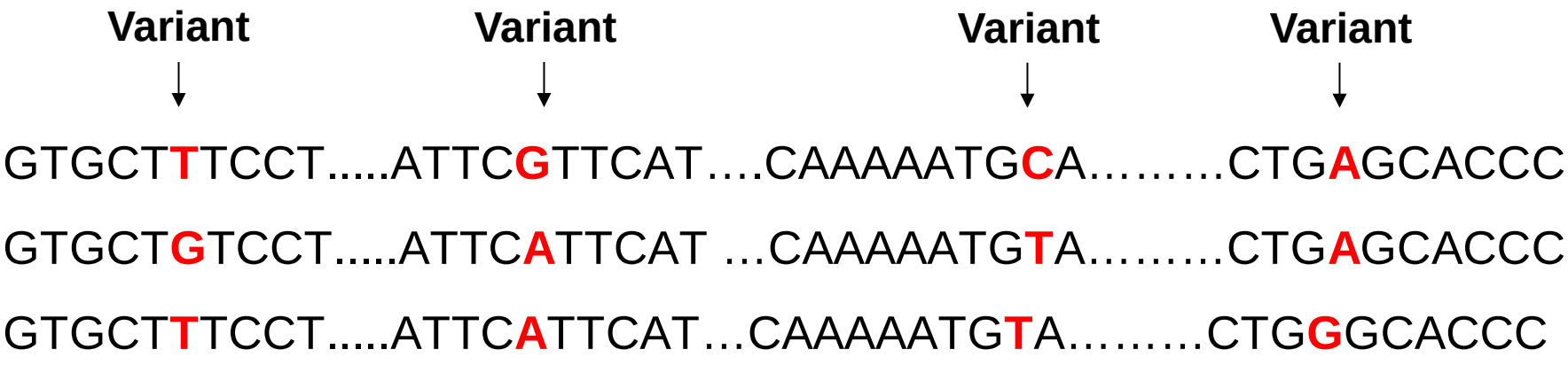
Methodology: Genomic DNA is extracted and tested for two Interleukin-1 polymorphisms. These polymorphisms are tested via polymerase chain reaction (PCR), followed by melt curve analysis.
Disclaimer: 1. OralDNA is not liable for any outcomes arising from clinician's treatment protocols and decisions. 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.

OralDNA Labs, Inc., 214 Overlook Circle, Suite 120, Brentwood, TN 37027 6155779055; Fax 6156272826 www.oraldna.com

Medical Director: Ronald McGlennen, MD

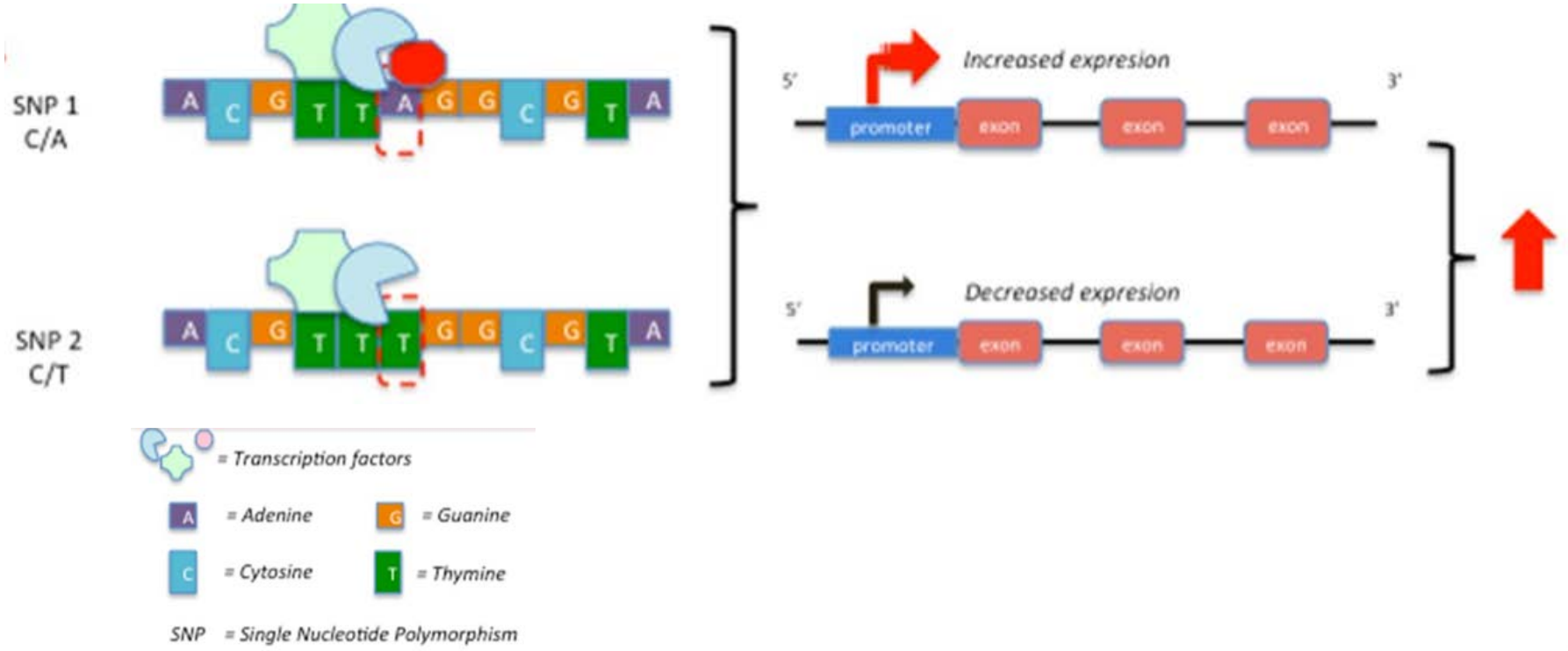
Chief Dental Officer: Thomas W. Nabors, DDS

Genetic Variation: The Basis of Human Diversity



Our ~~Genes~~ DNA Doesn't Say The Same Thing….

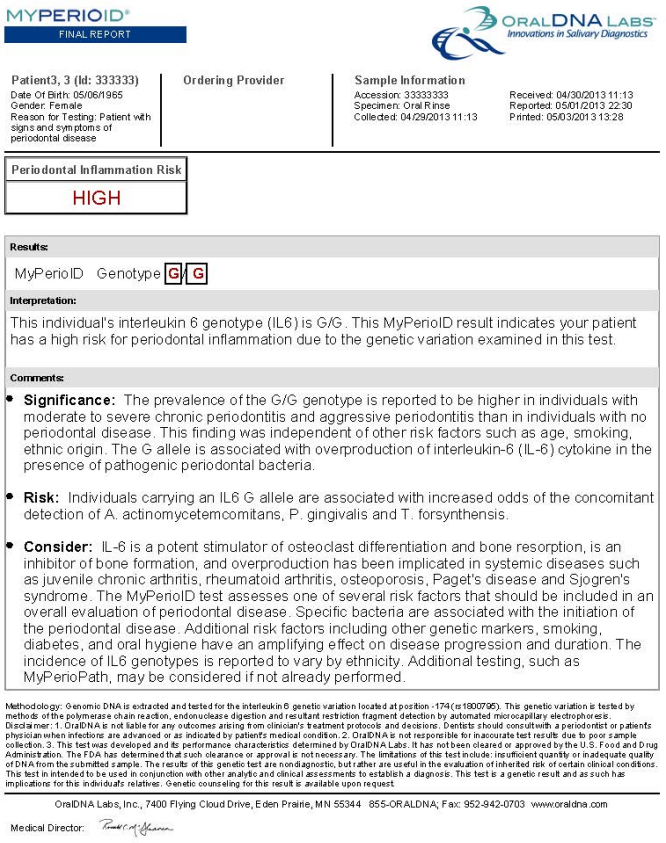
How SNPs Affect Gene Expression and Risk of Disease



Nucleotide polymorphisms i.e. SNPs typically reside outside of the coding region of a gene. The physiology of SNPs is in the effect on gene expression. Some SNPs result in higher levels of gene expression, and others, lower.

MyPerioID®: Genetic Assessment of The IL-6 Polymorphism

- MyPerioID® test for a single polymorphism (SNP) in the gene for Interleukin 6
- The interleukin 6 protein (cytokine) is a critical mediator of the primary inflammatory response to bacteria overgrowth
- Results are described as high, intermediate and low risk
  - High risk are persons with a heightened inflammatory response to bacteria
  - Heightened inflammatory response leads to associated bleeding, swelling and tissue destruction



MYPERIOID®
FINAL REPORT

ORALDNA LABS
Innovations in Salivary Diagnostics

Patient3, 3 (Id: 333333)
Date Of Birth: 05/06/1965
Gender: Female
Reason for Testing: Patient with signs and symptoms of periodontal disease

Ordering Provider

Sample Information
Accession: 33333333
Specimen: Oral Rinse
Collected: 04/29/2013 11:13
Received: 04/30/2013 11:13
Reported: 05/01/2013 22:30
Printed: 05/03/2013 13:28

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.

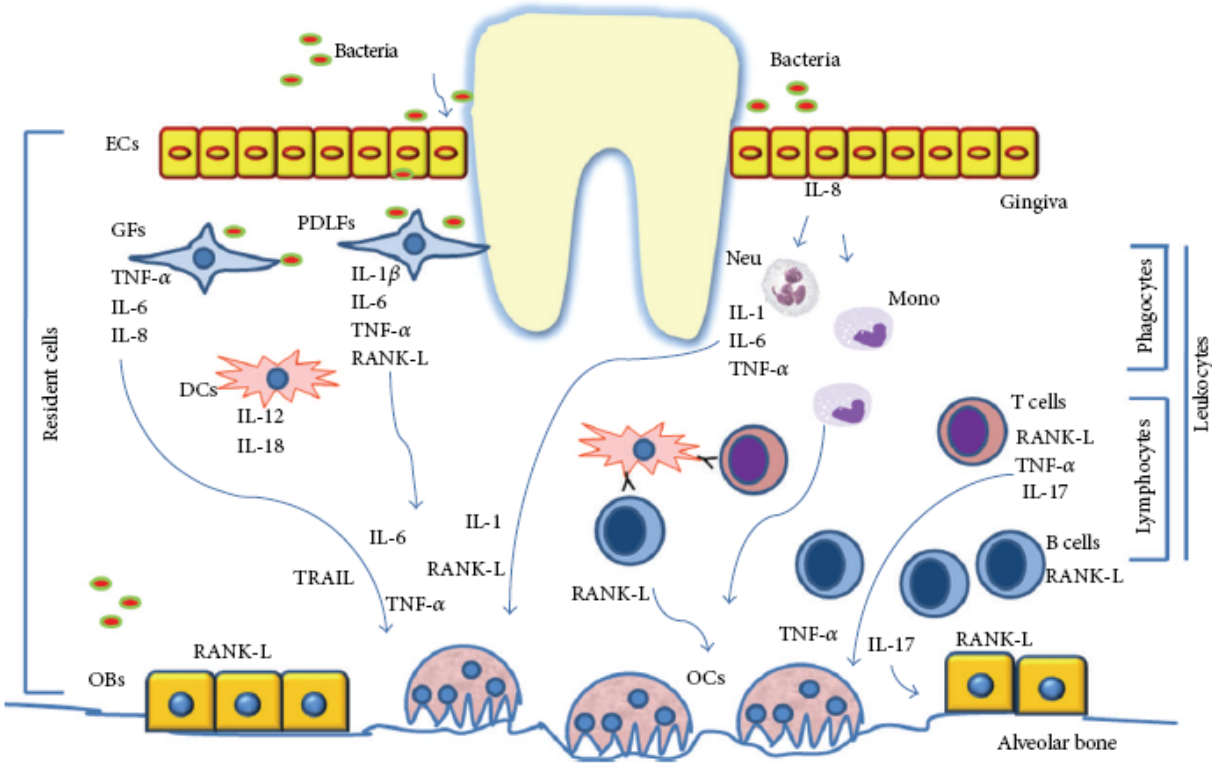
Methodology: Genomic DNA is extracted and tested for the interleukin 6 genetic variation located at position -174 (rs1800795). This genetic variation is tested by methods of the polymerase chain reaction, endonuclease digestion and resultant restriction fragment detection by automated microcapillary 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 non-diagnostic, 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.

OralDNA Labs, Inc., 7400 Flying Cloud Drive, Eden Prairie, MN 55344 855-ORALDNA; Fax: 952-942-0703 www.oraldna.com

Medical Director:

Proteins at Work: A Model of Periodontitis

- Like any biologic signaling pathway, there are multiple proteins involved
- Genetic mutations in any the genes involved in the signaling pathways can cause greater/less susceptibility to and severity of periodontal disease
  - Altered enzyme levels
  - Altered enzyme function/activity



Grano *et al*. Clinical and Developmental Immunology Volume 2013, Article ID 503754

Genetic Susceptibility to Periodontitis: Multi-genic Model

- Beyond the “Human Genome Project”, the research community is working through the subject of human diversity
  - Variations in human genomic sequence that impart to people differences in normal, and susceptibility to disease
  - Generally, the metrics of genetic susceptibility, due to any one gene marker, **is small**, and hence not observable clinically
  - In aggregate, however, the effect of multiple markers can be used to predict and prognosis disease outcomes

Calculating The Genetic Risk for Periodontitis

- There are a number of gene markers of risk for periodontitis
- Odds ratio is the standard method by which to calculate risk
  - How much more than normal is the risk of CP?
- The "net" effect of these gene markers may be:
  - Additive
  - Multiplicative
  - Positive and Negative

Marker	Range of Risk Multiple
IL1B-3954	.63-2.08
IL6-174	.55-2.46
IL10-592	.83-14.26
TNFa-308	.82-2.45
TLr4-299	.36-5.60

More than 70 differing gene markers have been demonstrated to have an associative role in CP

Candidate Gene Markers of Risk for CP

Gene	Coded protein
ACE	Angiotensin converting enzyme
CARD15(NOD2)	CaSpase recruitment domain-15 (NOD2)
CCR5	Chemokine receptor-5
CD14	CD-14
CTS C	Cathepsin C
ER2	Estrogen receptor -2
ET1	Endothelin-1
FBR	Fibrinogen
FCγRIIa	Fcγreceptor IIa
FCRIIb	Fcγreceptor IIb
FCRIIIa	Fcγreceptor IIIa
FCRIIIb	Fcγreceptor IIIb
FPR 1	N-Formy1 peptide receptor-1
G2m23	Immunoglobulin G2
HLADRB1	Human leukocyte antigen-DR
HLADQB1	Human leukocyte antigen-DQ
IFNGR1	Interferon γreceptor-1
IL1A	Interleukin-1α
IL1B	Interleukin-1β
IL1RN	Interleukin-1 receptor antagonist
IL2	Interleukin-2
IL3	Interleukin-3
IL4	Interleukin-4
IL6	Interleukin-6
IL10	Interleukin-10
IL18	Inteleukin-18
LTA	Lymphotoxin-α
MMP1	Matrix metalloproteinase-1
MMP3	Matrix metalloproteinase-3
MMP9	Matrix metalloproteinase-9
MPO	Myeloperoxidase
NAT2	N-acetyltransferase-2
PAI1	Plasminogen-activator-inhibitor-1
PGHS 1	Prostaglandin endoperoxidase synthase
RAGE	Receptor for advanced glycation end products
RANK	Receptor activator for nuclear factor kappa B
TGFB	Transforming growth factor- β
TIMP2	Tissue inhibitor of matrix metalloproteinase
TLR2	Toll-like receptor-2
TLR4	Toll-like receptor-4
TNFA	Tumor necrosis factor-α
TNFR2	Tumor necrosis factor receptor-2
VDR	Vitamin D receptor

- Reflecting the strong interest in finding the “causative” gene for CP, conventional wisdom suggests that there is not 1 gene, but likely many
- Is it possible to test a patient for the whole list?
  - yes

Do The CP Risk Markers Relate to Each Other?

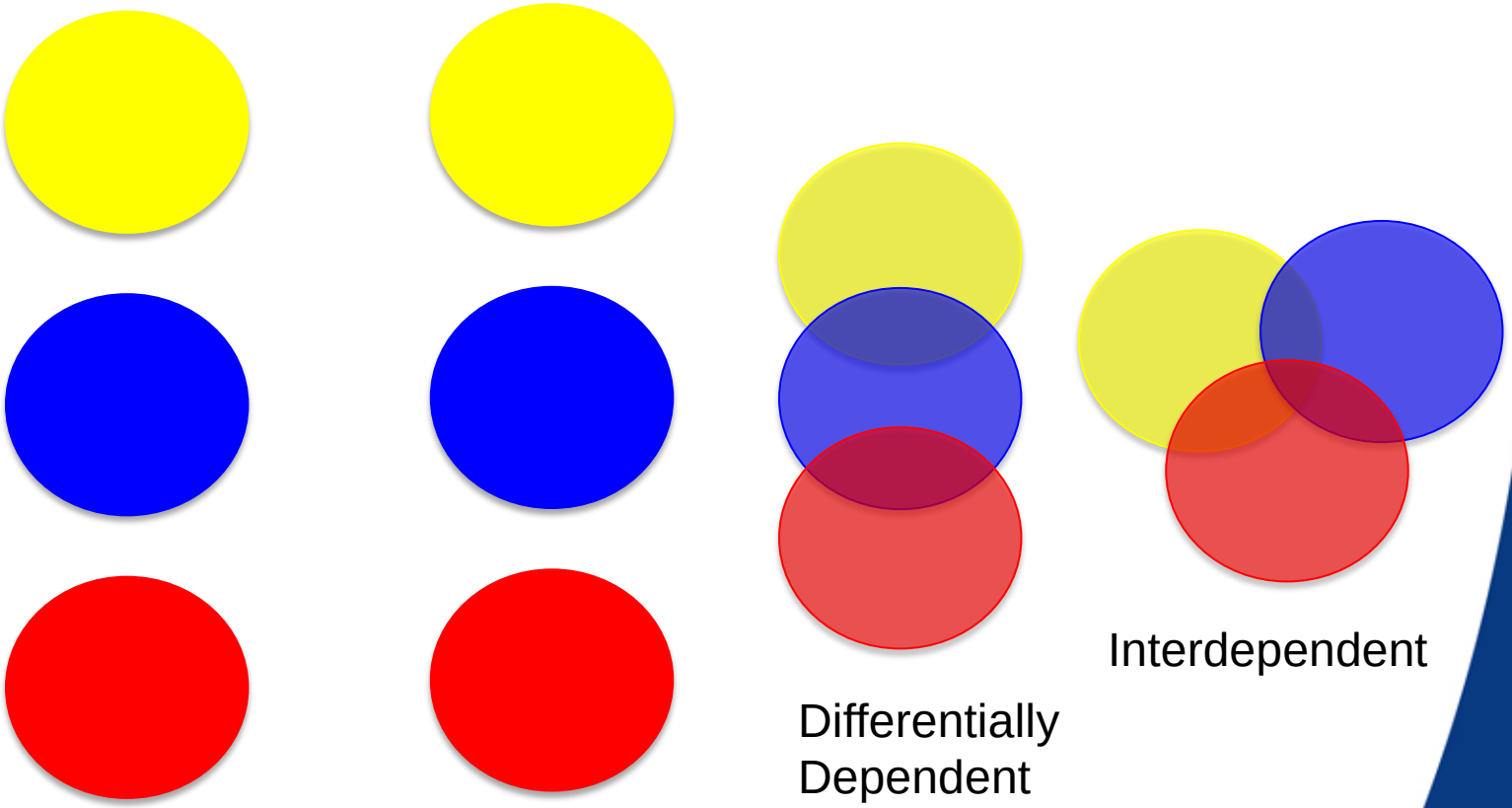
Prospective Gene Markers

A

B

C

How Do these Genes Relate to Each Other?



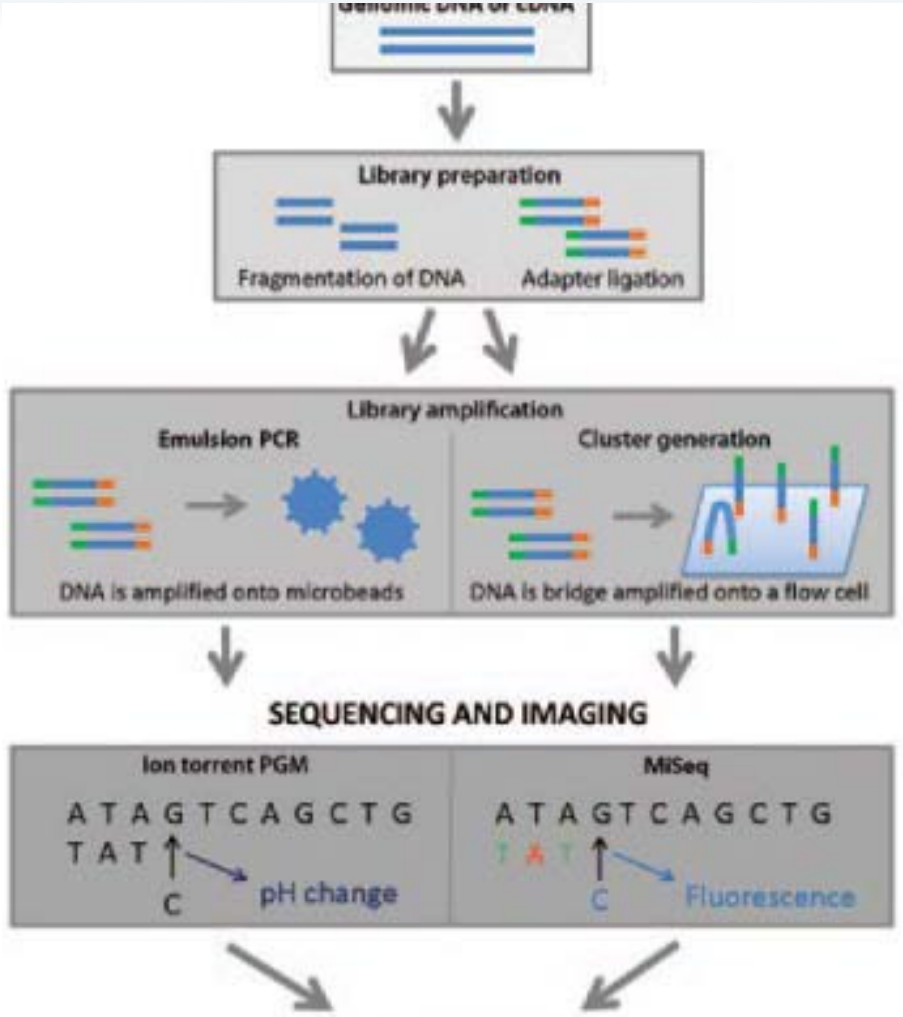
Independent

Differentially Dependent

Interdependent

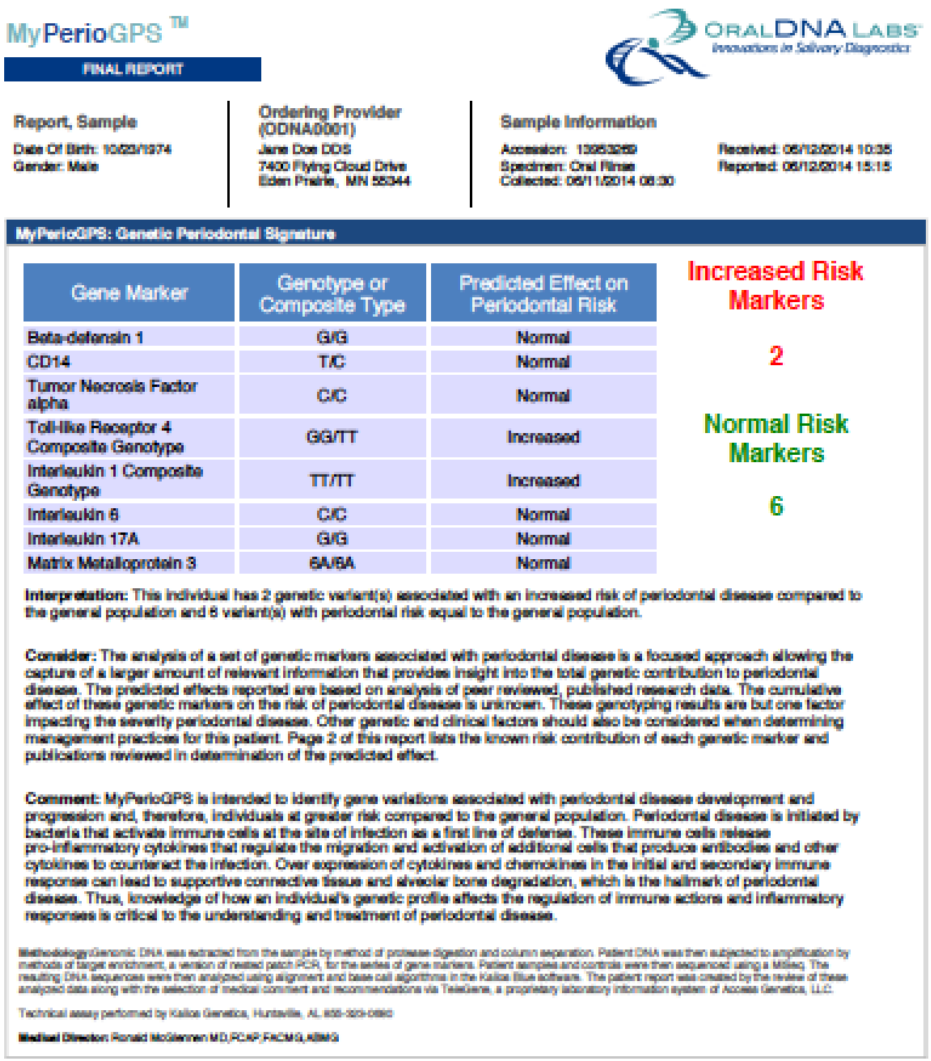
From PCR to Next Generation Sequencing

- Next Gen Sequencing
  - Lower cost and higher throughput alternative
  - Whole, and small genomes can be sequenced in about a day
  - Targeted sequencing allow the identification of disease causing mutation for diagnosis of pathological conditions
  - Target-Rich methods diminish testing challenges



Introducing MyPerioGPS®
Genetic Periodontal Signature

- The application of Next Generation Sequencing for salivary diagnostics
- MyPerioGPS® analyzes a series of 10 gene markers associated with the genetic risk of periodontitis
  - Results are reported as those markers with increase or normal risk
  - Each marker plays a unique and key role
    - Bone loss
    - Protection for bacterial infection
    - Vascular signs of inflammation
- MyPerio GPS® is highly unique to each patient and useful in the long term planning of patient care



MyPerioGPS ™

FINAL REPORT

ORALDNA LABS
Innovations in Salivary Diagnostics

Report, Sample
Date Of Birth: 10/23/1974
Gender: Male

Ordering Provider (ODNA0001)
Jane Doe DDS
7400 Flying Cloud Drive
Eden Prairie, MN 55344

Sample Information
Accession: 13953259
Specimen: Oral Rinse
Collected: 06/11/2014 08:30
Received: 06/12/2014 10:35
Reported: 06/12/2014 15:15

MyPerioGPS: Genetic Periodontal Signature

Gene Marker	Genotype or Composite Type	Predicted Effect on Periodontal Risk
Beta-defensin 1	G/G	Normal
CD14	T/C	Normal
Tumor Necrosis Factor alpha	C/C	Normal
Toll-like Receptor 4 Composite Genotype	GG/TT	Increased
Interleukin 1 Composite Genotype	TT/TT	Increased
Interleukin 6	C/C	Normal
Interleukin 17A	G/G	Normal
Matrix Metalloprotein 3	6A/6A	Normal

Increased Risk Markers
2

Normal Risk Markers
6

Interpretation: This individual has 2 genetic variant(s) associated with an increased risk of periodontal disease compared to the general population and 8 variant(s) with periodontal risk equal to the general population.

Consider: The analysis of a set of genetic markers associated with periodontal disease is a focused approach allowing the capture of a larger amount of relevant information that provides insight into the total genetic contribution to periodontal disease. The predicted effects reported are based on analysis of peer reviewed, published research data. The cumulative effect of these genetic markers on the risk of periodontal disease is unknown. These genotyping results are but one factor impacting the severity periodontal disease. Other genetic and clinical factors should also be considered when determining management practices for this patient. Page 2 of this report lists the known risk contribution of each genetic marker and publications reviewed in determination of the predicted effect.

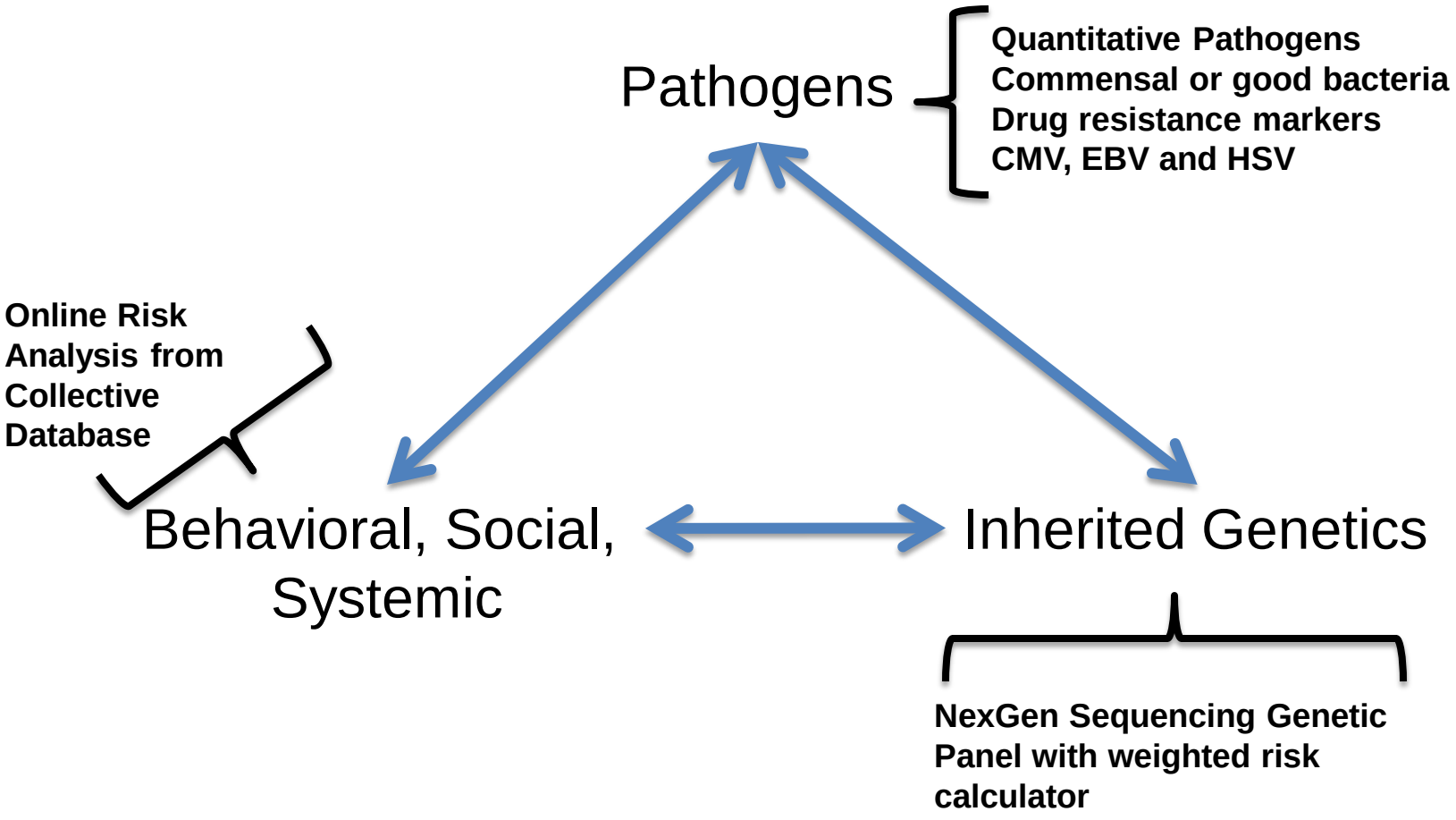
Comment: MyPerioGPS is intended to identify gene variations associated with periodontal disease development and progression and, therefore, individuals at greater risk compared to the general population. Periodontal disease is initiated by bacteria that activate immune cells at the site of infection as a first line of defense. These immune cells release pro-inflammatory cytokines that regulate the migration and activation of additional cells that produce antibodies and other cytokines to counteract the infection. Over expression of cytokines and chemokines in the initial and secondary immune response can lead to supportive connective tissue and alveolar bone degradation, which is the hallmark of periodontal disease. Thus, knowledge of how an individual's genetic profile affects the regulation of immune actions and inflammatory responses is critical to the understanding and treatment of periodontal disease.

Methodology:Genomic DNA was extracted from the sample by method of protease digestion and column separation. Patient DNA was then subjected to amplification by methods of target enrichment, a version of nested patch PCR, for the series of gene markers. Patient samples and controls were then sequenced using a MiSeq. The resulting DNA sequences were then analyzed using alignment and base call algorithms in the Kallos Blue software. The patient report was created by the review of these analyzed data along with the selection of medical comment and recommendations via TeleGene, a proprietary laboratory information system of Access Genetics, LLC.

Technical assay performed by Kallos Genetics, Huntsville, AL 855-923-0880

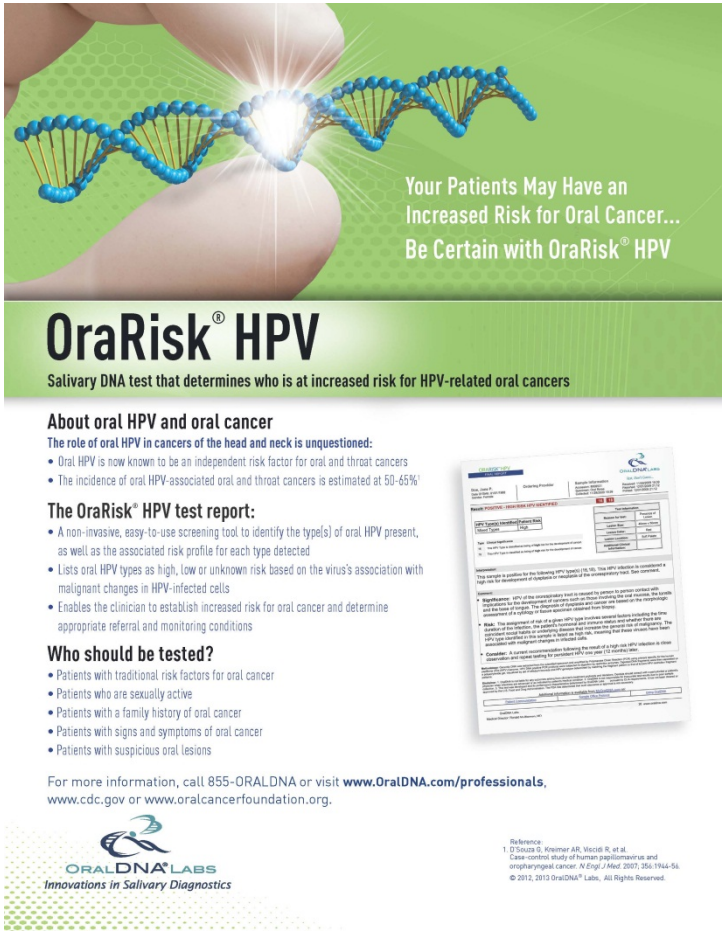
Medical Director: Ronald McGlennen MD,FCAP,FACMG,ABMG

Future Tests Offering for Management of Periodontitis



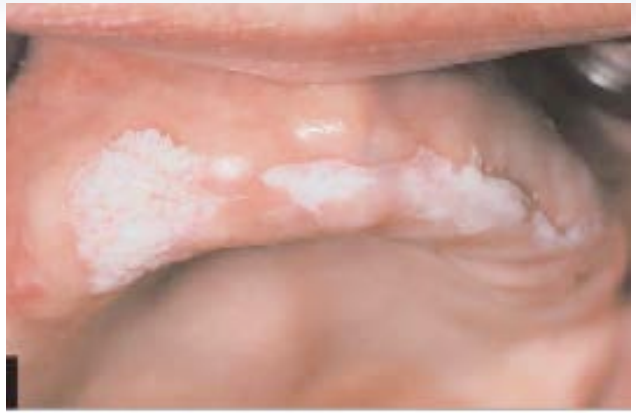
HPV and The Clinical Search for Oral Cancer

- Management of diseases of primary concern to dentists is the opportunity to use the clinical laboratory to enhance the interface between dentistry and other medical specialties
- The application of testing for oral HPV infection is one such example
  - HPV is first an infection-**screening** for cancer risk
  - HPV is associated with a common cancer-**diagnosis**
  - HPV in oral tumors is a good **prognostic** feature

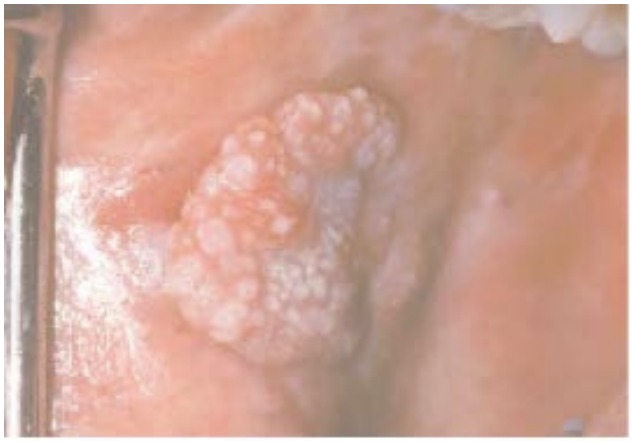


HPV Associated Oral Lesions

- The role for HPV in cancers of the head and neck is unquestioned
  - The prevalence of HPV infection in the oral cavity is unknown but is approximated to be 18-25%
  - The incidence of HPV associated oral cancers is estimated at 50-70% of all oral cancers
  - Current testing modalities identify HPV in approximately 70% of oral epithelial lesions



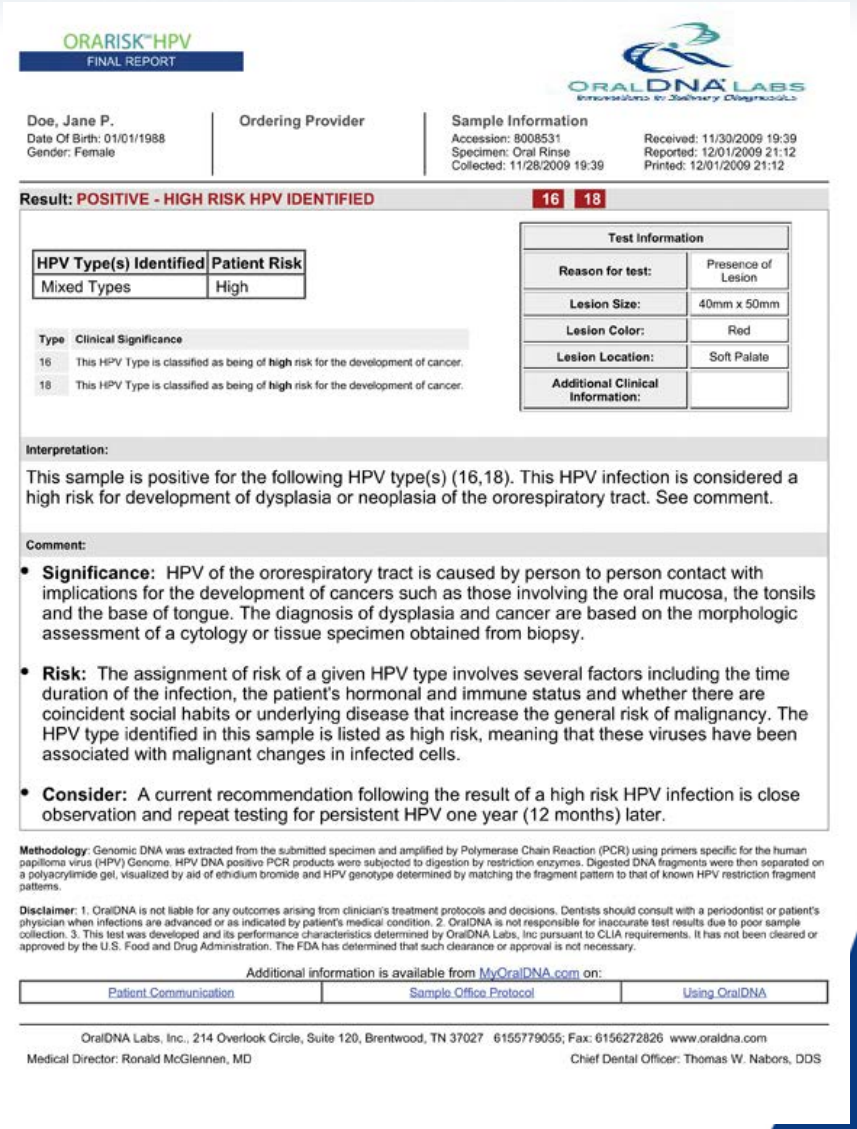
Oral leukoplakia



Squamous cell carcinoma

OraRisk® HPV: Type Specific Detection of Human Papillomavirus from an Oral Rinse

- HPV is now known to be associated with oral dysplasia and cancers as well as in other sites of the head and neck
- As is true in the case of the genital tract, knowing the type of HPV is imperative
- HPV are listed as high and low risk, based on the likelihood of transforming cells
  - Most infections in the mouth are high risk.



ORARISK™HPV
FINAL REPORT

ORALDNA LABS
Innovations in Salivary Diagnostics

Doe, Jane P.
Date Of Birth: 01/01/1988
Gender: Female

Ordering Provider

Sample Information
Accession: 8008531
Specimen: Oral Rinse
Collected: 11/28/2009 19:39
Received: 11/30/2009 19:39
Reported: 12/01/2009 21:12
Printed: 12/01/2009 21:12

Result: POSITIVE - HIGH RISK HPV IDENTIFIED 16 18

HPV Type(s) Identified	Patient Risk
Mixed Types	High

Type	Clinical Significance
16	This HPV Type is classified as being of **high** risk for the development of cancer.
18	This HPV Type is classified as being of **high** risk for the development of cancer.

Test Information	
Reason for test:	Presence of Lesion
Lesion Size:	40mm x 50mm
Lesion Color:	Red
Lesion Location:	Soft Palate
Additional Clinical Information:	

Interpretation:

This sample is positive for the following HPV type(s) (16,18). 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 and the base of tongue. The diagnosis of dysplasia and cancer are based on the morphologic assessment of a cytology or tissue specimen obtained from biopsy.
- **Risk:** The assignment of risk of 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 these viruses have been associated with malignant changes in infected cells.
- **Consider:** A current recommendation following the result of a high risk HPV infection is close observation and repeat testing for persistent HPV one year (12 months) later.

Methodology: Genomic DNA was extracted from the submitted specimen and amplified by Polymerase Chain Reaction (PCR) using primers specific for the human papilloma virus (HPV) Genome. HPV DNA positive PCR products were subjected to digestion by restriction enzymes. Digested DNA fragments were then separated on a polyacrylimide gel, visualized by aid of ethidium bromide and HPV genotype determined by matching the fragment pattern to that of known HPV restriction fragment patterns.

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, Inc 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	Sample Office Protocol	Using OralDNA

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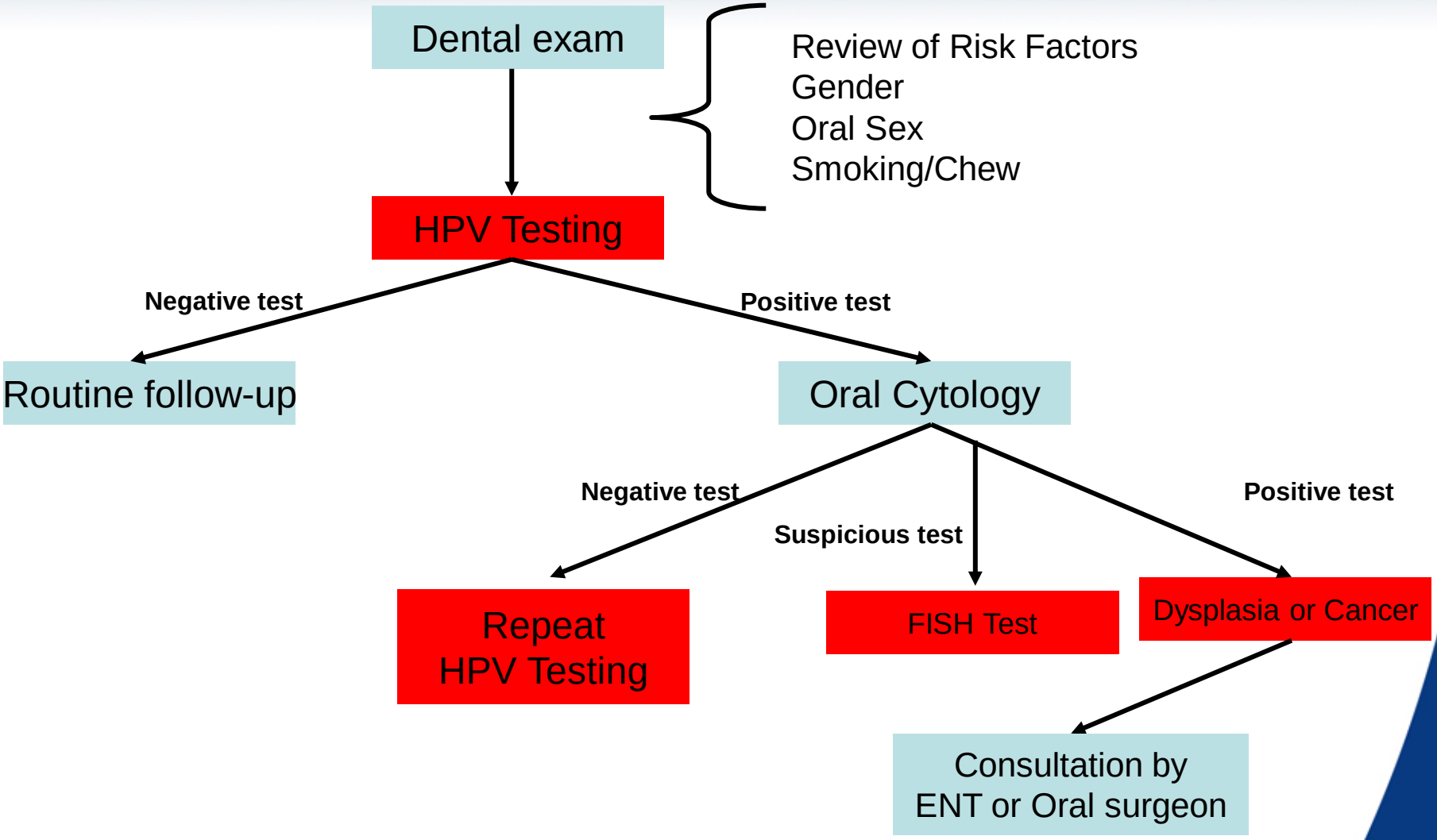
A Model to Screen for Oral Cancer



Summary

- The routine use of gene based testing is routine and part of the management of every patient with periodontitis. Such tests are:
  - Objective
  - Useful in selection of therapy
  - Predictive
- Specifically:
  - Test for the offending pathogens
  - Re-test consequent to treatment to assess the effect on bacteria
  - Test for inherited genetic markers in consideration of the long-term plan for each patient

Salivary Diagnostics and the AAOSH Mission

- The advent of the salivary diagnostics offers the dentist and physicians alike with results that are objective, reliable and actionable

- Valuable in the assessment of health and disease in other organ systems

- This lecture has been a preview of the role of these tests in the assessment of periodontitis, equal time could be spent on the matters of
  - Preventative cardiology
  - Metabolic syndromes
  - Disease of immunity

OralDNA® Labs

- **DNA(bacterial)**
  - **MyPerioPath®** establishes bacterial risk and can help guide therapy based on causation
- **DNA (genetic)**
  - **MyPerioID® and MyPerioGPS®** establishes genetic risk and can help guide therapy based on genetics **(GPS genetic panel coming soon)**
  - **DNA DrugMap®** allows for personalizing prescription and dosing choices
- **DNA (viral)**
  - **OraRisk® HPV** identifies HPV status (separate risk factor for oral cancers)

Thank You!

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