Salivary Diagnostics

IEPSC Member Presentation

Nov. 5th, 2010

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LLAMAS

Traditional Perio Screening

- Traditional Diagnosis of Periodontal Disease
  - Measurement of the past damage
  - Want to look at ways to improve diagnostic abilities
- Progression of the patients periodontal disease maybe more advanced than we think at time of evaluation
- Can be a timely process
- Can be painful to patient
- Periodontal screening does not directly tell us if the disease process is still active or arrested
- Still standard of care and necessary

Salivary Diagnostics: Historical Perspective

- What can saliva tell us?
- Salivary Diagnostics
  - 1960's: Irwin Mandel D.D.S.
    - Difficulty with sampling and storage which led to inconsistent results
  - 1993: Malamud et. al established standards for saliva collection
  - Throughout 1990's saliva used in discovering biomarkers for:
    - Caries, HIV, cancers, diabetes, heart disease, autoimmune disorders as well as monitoring legal/illegal drug usage
    - Large focus has been on detecting oral cancers

Salivary Diagnostics: Historical Perspective

- Technology continually advances but at a slow pace
- National Institute of Dental and Craniofacial Research
  - Dr. David Wong and colleagues

Point Of Care Testing

- POCT is defined as “diagnostic testing at or near the site of patient care.”
  - Convienience
  - Immediate results
  - Cheaper, smaller, faster, and smarter POCT devices have increased the use of POCT approaches by making it cost-effective for disease identification
- Glucose testing for diabetics
- High Demand Worldwide: identify health risks and illnesses which can help eliminate inappropriate treatment

What Can Saliva Tell Us?

- Saliva is considered a mirror of the body
- Previous drawback to using saliva
  - Informative analytes in saliva are generally lower in amounts than in serum
  - Saliva has 10^{-12} picograms of protein: lower concentration compared to other body fluids
- Use of saliva for disease diagnostics

Tests Available to Dentists

- Sampling Gingival Crevicular Fluid
  - Results may differ depending on site in oral cavity that was sampled
  - Slow/Hard process
- Blood Testing: identify serum levels of C-reactive protein
  - Serum level of CRP not specifically increased due to periodontal disease alone
  - Painful to the patient
- Buccal Swabs
  - Easy to obtain
  - Reliable results?

Advantages of Salivary Diagnostics

- Painless, Non-Invasive: lowers anxiety and may increase patient acceptance to testing
- Safer than blood
- Obtained at Low Cost
- Quick results
- Identifies specific susceptibility for each patient and pathogens involved
- Proactive: treatment can be modified based on clinical and biological inflammatory factors
  - Can move patients into therapeutic stages earlier
  - Eliminates “one size fits all” treatment philosophy

Advantages of Salivary Diagnostics

- Educates patient on oral-systemic link and may lead them to seek appropriate medical care
- Improved communication and case acceptance
- Possibly advantage for use in our young adult population

Technology Available

- PCR: polymerase chain reaction
- Lateral Flow Immunochromotography: example is OTC pregnancy test
- Protein Microarray: Laser Conofocal Excitation and Charged Coupled Device (CCD)
- Lab on a Chip: (LOC) based on advanced nanotechnology
  - POCT

Oral DNA Labs

- Three Tests Available:
  - MyPerioPath
    - Pathogen analysis of type and amount of pathogens
  - MyPerioID PST
    - Genetic testing for susceptibility of periodontal disease
  - OralRisk HPV Testing
  - All are collected in same manner
    - Rinse with saline solution for 30 seconds and expectorate into collection tube and mail to lab

Oral DNA Labs

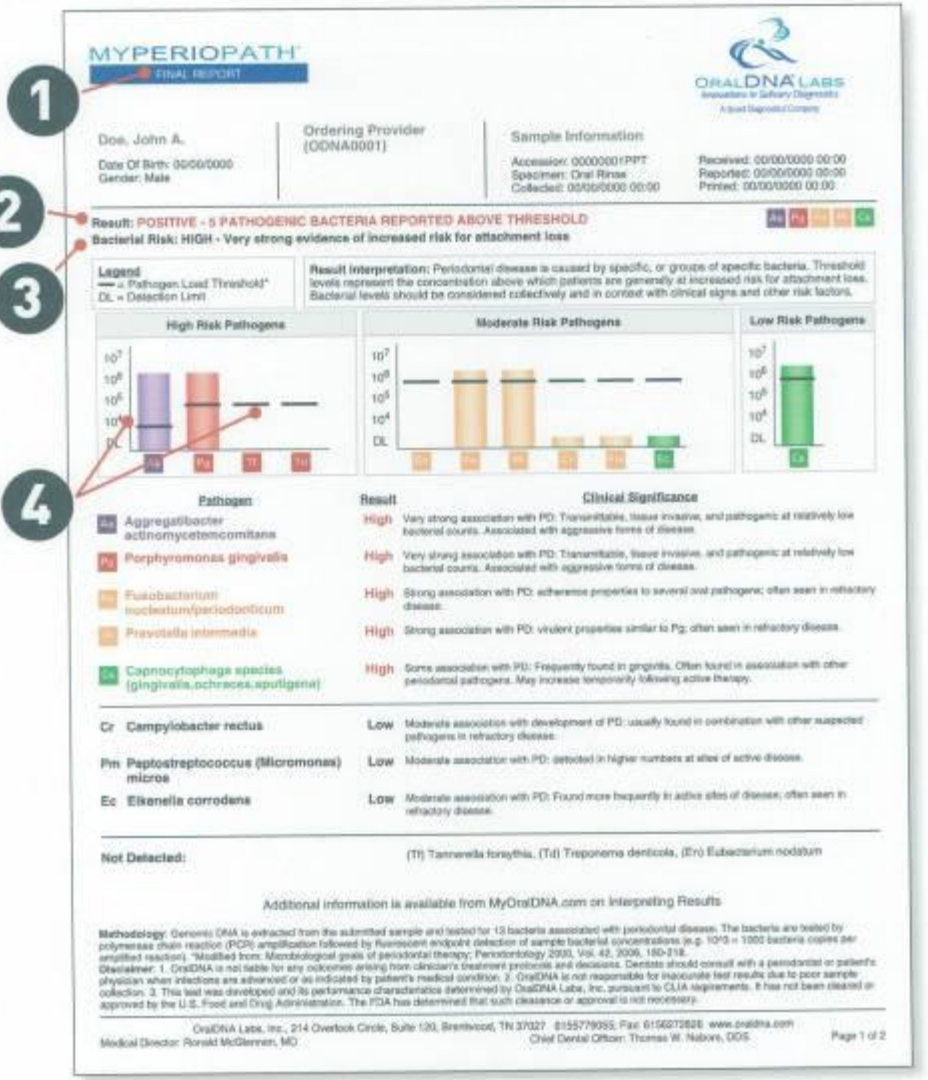
- MyPerioPath:
  - Bacterial DNA test: tests for 11 bacteria associated as key markers of periodontal disease
    - PCR- polymerase chain reaction amplification

Oral DNA Labs

- Who Might benefit from MyPerioPath test?
  - Patients with signs/symptoms of periodontal disease
  - PD > 4mm with BOP/Exudate
  - Little to no response to conventional therapy: ScRP and periodontal maintenance
  - Patients with history of CVD and Smokers

MyPerioPath®
INTERPRETING RESULTS

ORALDNA LABS



MYPERIOPATH®

FINAL REPORT

ORALDNA LABS

Doe, John A.

Date Of Birth: 00/00/0000
Gender: Male

Ordering Provider
(ODNA0001)

Sample Information

Accession: 0000000rPPT
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

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
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.
Fusobacterium nucleatum/periodonticum	High	Strong association with PD: adherence properties to several oral pathogens; often seen in refractory disease.
Prevotella intermedia	High	Strong association with PD: virulent properties similar to Pg; often seen in refractory disease.
Cs Capnocytophaga species (gingivalis,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

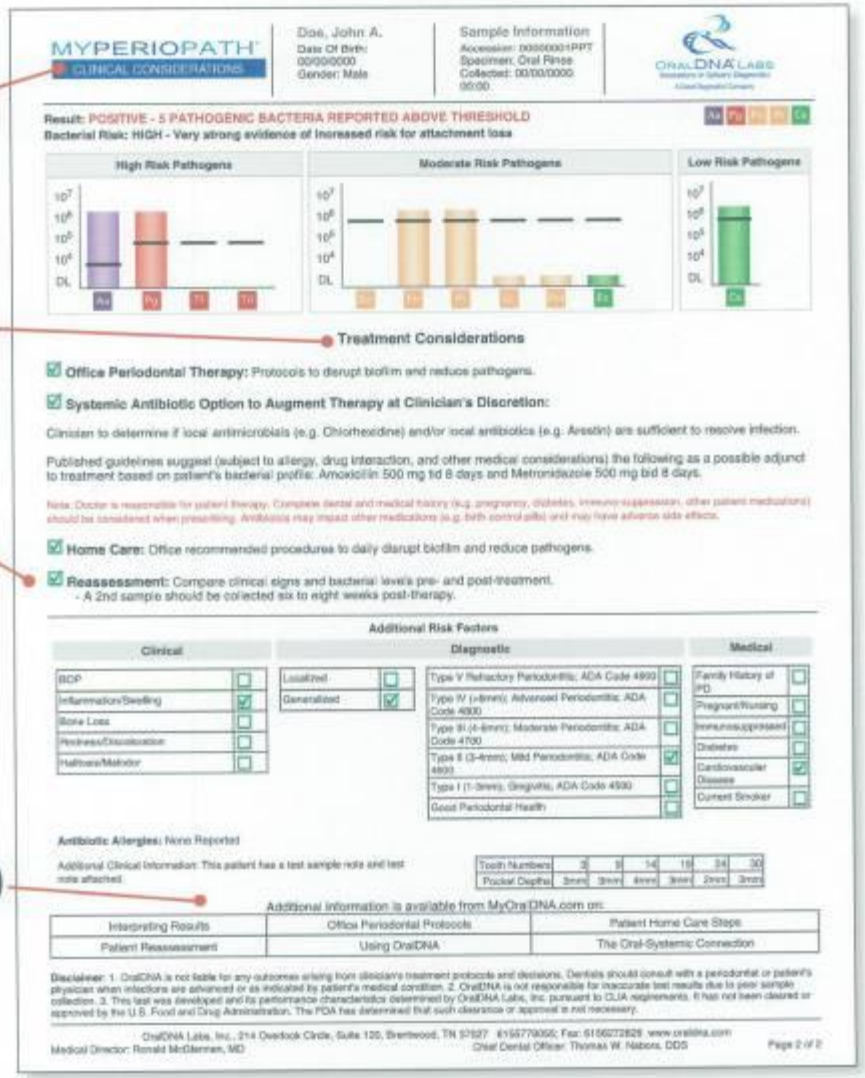
Methodology: Genomic DNA is extracted from the submitted sample and tested for 13 bacteria associated with periodontal disease. The bacteria are tested by polymerase chain reaction (PCR) amplification followed by fluorescent endpoint detection of sample bacterial concentrations (e.g. 10^3 = 1000 bacteria 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. 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.

OralDNA Labs, Inc., 214 Overlook Circle, Suite 120, Brentwood, TN 37027 615577905S; Fax: 6156972626 www.oraldna.com
Medical Director: Ronald McGlennen, MD Chief Dental Officer: Thomas W. Nabors, DDS

Page 1 of 2

1. Page 1 emphasizes the bacterial profile
2. A "Positive" result indicates bacterial pathogens detected above threshold
3. "Bacterial Risk" Risk of disease progression based on specific bacterial pathogens.
4. "Pathogen Load Threshold" The concentration above which patients are generally at increased risk of attachment loss/disease progression (represented by the black lines)

ORALDNA LABS
Innovations in Salivary Diagnostics
A Quest Diagnostics Company



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

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

Aa Pg Tf Pi Ec

High Risk Pathogens | Moderate Risk Pathogens | Low Risk Pathogens

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

Aa Pg Tf Td | Cs Fn Pi Vd Pm Ec | 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	☐	Family History of PD	☐
Inflammation/Swelling	☑	Generalized	☑	Pregnant/Nursing	☐
Bone Loss	☐	Type V Refractory Periodontitis; ADA Code 4999	☐	Immunosuppressed	☐
Redness/Discoloration	☐	Type IV (>6mm); Advanced Periodontitis; ADA Code 4900	☐	Diabetes	☐
Halitosis/Malodor	☐	Type III (4-6mm); Moderate Periodontitis; ADA Code 4700	☐	Cardiovascular Disease	☑
		Type II (3-4mm); Mild Periodontitis; ADA Code 4800	☑	Current Smoker	☐
		Type I (1-3mm); Gingivitis; ADA Code 4500	☐		
		Good Periodontal Health	☐		

Antibiotic Allergies: None Reported

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

Tooth Numbers	3	8	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

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Medical Director: Ronald McGlennen, MD Chief Dental Officer: Thomas W. Nabors, DDS

Page 2 of 2

5 Page 2 emphasizes clinical considerations

6 Options to reduce bacterial risk

7 MyPerioPath® can be used to assess response to therapy

8 Links to additional information at MyOralDNA.com

Oral DNA Labs

- MyPerioID PST:
  - Molecular test for mutations of IL-1
    - Identifies an individuals predisposition for over expression of inflammation and risk for more severe periodontal disease

Oral DNA Labs

- Who Might benefit from MyPerioID test?
  - Patient with family history of periodontal disease
  - Immunocompromised patients
  - Little to no response to conventional therapy: ScRP and periodontal maintenance
  - Adolescents

ORALDNA LABS

MYPERIOID PST

FINAL REPORT

ORALDNA LABS

1, Patient	Ordering Provider	Sample Information	
Date Of Birth: 00/00/0000 Gender: Male	John A. Doe 214 Overlook Circle, Suite 120 Brentwood, TN 37027	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

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 single base extension detection.

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 Interleukin Genetics 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 (877) 577-9055; Fax: (615) 627-2826 www.oraldna.com
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Oral DNA Labs

- OraRisk HPV
  - screening tool to identify the type(s) of HPV and associated risk of developing cancer
- Who might benefit?
  - Patients who are sexually active
  - Patients with signs and symptoms of oral cancer
  - Patients with traditional risk factors for oral cancer
  - Patients with suspicious oral lesions

ORARISK™ HPV
FINAL REPORT

ORALDNA LABS
Innovations in Salivary Diagnostics
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Patient8, 8 (Id: 4856854)
Date Of Birth: 12/15/1982
Gender: Female

Ordering Provider

Sample Information
Accession: HPVIDORAL32463
Specimen: Oral Rinse
Collected: 11/17/2009 21:52
Received: 12/01/2009 21:52
Reported: 12/12/2009 00:01
Printed: 12/12/2009 00:01

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 of **high** risk for the development of cancer.

Clinical Information	
Reason(s) for test:	Presence of Lesion
Lesion Size:	8mm x 88mm
Lesion Color:	Red
Lesion Location(s):	Posterior Pharyngeal Wall
Additional Clinical Information:	N/A

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) have 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 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.
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Additional information is available from MyOralDNA.com on:

Patient Communication	Possible Office Workflow	Using OralDNA

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Oral DNA Labs

- MyPerioID and MyPerioPath relationship
  - Modification of treatment
    - Positive Test: more aggressive approach
    - Standard protocol in all patients with diagnosed periodontal disease: ScRP and OHI
    - Local Delivery or Systemic Antibiotics at discretion of clinician
      - Arrestin, Chlorohexideine, Amoxicillin/Metronidazole
    - Reassessment at 6-8 weeks: possibly test again?

How much will this cost?

- All Oral DNA Lab tests: distributed through Henry Schein
  - 1 box gets you 12 individual tests for $199.99
    - Each individual test will give you results for both MyPerioPath and MyPerioID
    - $199.99/12 = $16.66 per test plus analysis fee
  - Each test then requires analysis: $99 for one or $198 for both Path/ID
  - HPV test includes 12 individual test for $199.99
  - Analysis of HPV test is $70

Ethical Perspective

- Scope of practice in Dentistry:
  - As technology advances and more tests become available using saliva how far do we go?
  - Paradigm Shift
- Informing our patients
  - Disclosure of enzymes, proteins, biomarkers, gene variants that indicate predispositions for various cancers and diseases of the body, not just the oral cavity
- Grounds for insurance companies to deny, limit or cancel coverage
- Since tests are based on saliva, do they solely belong in the domain of the dentist?

What's Next?

- Survey conducted by ADA in 2007 "suggested that dentists believe screening for medical conditions is important and that they generally are willing to do it."
- Do we agree?
- Lab on a Chip (LOC)
  - Oral Fluidic NanoSensor Test (Wong, Lee and Garon at UCLA)
    - A handheld, automated device used to detect multiple salivary proteins and nucleic acid targets to be used in POCT
  - Access to care

Potential Game Changer?

- 2007 National Health Interview Survey found 60.3% of adults in U.S. reported a dental visit during the past 12 month
  - As dentists we would like to see a higher percentage of re-care exams annually but this is still better compliance than visits to primary care physicians
- Does this put us in a position to potentially screen for more than diseases of the mouth with saliva diagnostics?

Potential Game Changer?

- Our education? Education of future dentists?
- Benefit to the patient by providing this service?
- What if we don’t act on this and utilize the technology?
  - Will the health profession take charge?