CDA

Novel Saliva Test

Oral-Based Techniques

New Technology

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salivary diagnostics.

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DEPARTMENTS

FEATURES

Alan L. Felsenfeld, DDS

It's Not Your Father's Dentistry

Recently, for reasons that are not entirely clear to me, or perhaps in a fit of nostalgic reflection, I pulled my old dental school yearbook from the shelf, dusted it off, and began to look at pictures of my classmates. A number of revelations were apparent. There is no reason to comment on the hair, eyeglasses, and dress standards of those bygone years. Suffice it to say, that at the time, they were all in style.

It was a bit depressing that I had no idea where many of my classmates were practicing or if they had retired. Most astonishing was the overall appearance of the class. We were a group of white men with the occasional Asian, and far, far fewer women, blacks and Hispanics. No American Indians were in my group. Classes that preceded me had even less diversity, but not for prejudicial reasons. Rather, the applicant pool at my school was reflected in the class picture. While it might be unfair to extrapolate, it would not be surprising to see similar picture arrays at our other California schools at that time. We all attended class and took copious notes. Most of us went into general dental practice; a few of us specialized or took postdoctoral general practice programs.

A comparative look at the entering class picture at my school some 35 years later reveals an entirely different mix. Gone is the predominant white male face from the picture. There are numerous Asians, and more than half of the photos are of women. The number of blacks, Hispanics and American Indians remains low but has increased from years ago. The applicant pool in those areas has increased substantially over the years. Couple that with the increasing number of dental students from other countries, either by birth or their parents' immigration, and there is a different look to the class. Frequently, unless a quiz is given in class, less than all of the students are in attendance. Note pools and computer dissemination of lecture material are popular. In our institution, close to 70 percent of the graduates either enter specialty training or do an AEGD or GPR program.

This is a finding that is not only prevalent at our school but appears to be evident nationally. Recent analysis of entering dental students throughout the United States shows these are emerging and consistent trends. Slightly less than one-half of first-year students are women — a number that has been stable for the past five years. This accounts for approximately 16 percent of the active practicing dentists and is a growing portion. On the state level, this translates into about 44 percent of all new CDA members in the past three years being female.

Black, Hispanic and American Indian enrollees have not grown proportionately but have increased from the early 1970's.[1] According to American Dental Association data, Caucasian dentists are 86 percent and Asians represent 7 percent of all dentists. The remainder nationally includes blacks at 3.5 percent and Hispanics at 3.3 percent. Less than 1 percent of all dentists are Native American.[2] First-year dental school enrollment for all underrepresented minorities has increased significantly from

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the earlier years on a national level but has leveled off in the past 15 years. On a national level, approximately 20 percent of all first-year students are Asian, with slightly less than 6 percent for each of the black and Hispanic groups.[3]

Equally important, perhaps more important, are the attitudes these young students bring to their careers. There has been a proliferation of courses and presentations in the area of changing social mores. The gurus have begun to classify all of us by our age and value systems. Most of us who are in the mature dental practice aspect of our careers are in the Traditionalist or Baby Boomer generations, with a series of values and ideals that are somewhat incongruent with those of the younger dentists, known as Generation X, or for entering students, the Millennials.

Traditionalists have expectations of long careers with minimal outside interferences and clearly defined goals. The Baby Boomers have similar values but desire intermittent recognition and self-actualization. In contrast, Generation X-ers do not have the same concept of devotion to one career or job, and are more likely to shift their focus from time to time. Family values and raising children are a high priority for them as is instantaneous reward and continuous feedback. The up-and-coming Millennials are truly multitasking individuals with commitment to numerous activities, work being only one of them.[4]

This generational divergence was hammered home in some recent interviews I conducted for our residency program. Out of 15 applicants, it was surprising to learn that when asked why they wanted to come to Los Angeles, about six or seven of them talked about lifestyle for their family as a priority. A similar attitude was revealed when I asked a potential dental student what he wanted to do after he finished dental school. His reply was to go practice, make a good living, and raise a large family.

There are several implications to all of this information. First, we, as a profession, can expect changes in our educational system to accommodate the increasing diversity of our students if we are to be effective in completing our responsibility to educate them. Those of us who expect younger dentists to pay large sums of money to purchase our practices as we retire may be surprised to find that the upcoming generation may not be as interested in owning their own practices, or even being a solo practitioner as we were so many years ago. The California Dental Association, the American Dental Association, and the profession need to appreciate the increasing diversity of not only the individuals by gender, ethnicity, or country of origin, but by attitudes toward the profession and organized dentistry. If we are to maintain ourselves, we need to be continually assessing if we are providing the services our new generations need or perceive as important.

During the past 35 years, dentistry has changed and so have dentists. The CDA is sensitive to these issues and is proactive in this arena. This needs to continue to be a high priority for organized dentistry if we intend to be a viable force in the lives of young dentists in the future. CDA

References / 1. Weaver RG, Ramanna, MS, et al, U.S. dental school applicants and enrollees: 2003 and 2004. *J Dent Educ* 69(9):1064-72, September 2005.

2. American Dental Association, Bureau of Health Professions, HRSA.

3. American Dental Education Association, Financing a dental education: Implications for URMs and the W.K. Kellogg/ADEA access to dental careers (ADC) grant. Forty-third annual deans' conference, 2001.

4. Lancaster LC, Stillman D, Tips for working with people at work. Humanresourcesabout.com, 2006.

Comments, letters and questions can be addressed to the editor at alan.felsenfeld@cda.org.

Illustration: Dan Hubig

Dentists Encouraged to Consult Reliable Sources in Potential Influenza Pandemic

The American Dental Association has issued a report on the possible global spread of severe influenza and how dentists can be responsive to such an event in the United States.

"The threat of an influenza pandemic is real, and prediction of a pandemic's onset or immediate containment remains impossible," said Albert H. Guay, DMD, ADA chief policy adviser.

"Yet, this is not a reason for hysterics or panic. Instead, I urge dentists to pay attention to health reports from around the world, and to look for balanced information from reliable sources — not from alarmist opinions."

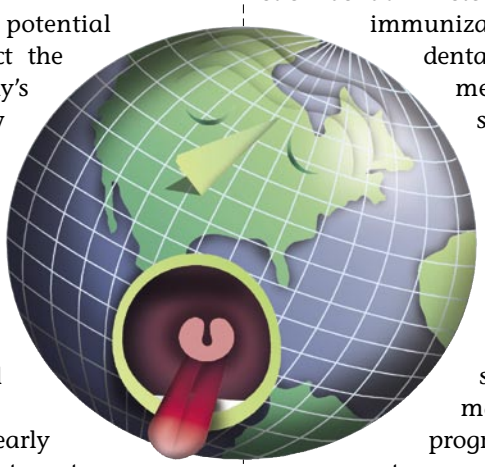
In his report, Guay outlined the historical characteristics and typical progression of an influenza pandemic. In

"Dentists may need to play a hands-on and vital role to counteract the spread of disease and safeguard the public health."

ALBERT H. GUAY, DMD

discussing the federal government's strategies to handle a future event, Guay clearly noted that no predetermined plan will be absolutely the best for whatever situation may occur, since information about the exact nature of a pandemic must await its emergence. Nonetheless, a well-developed plan will allow a mechanism to establish for the most rapid response, Guay said.

"How badly a potential pandemic might affect the United States is anybody's guess, but we do know that our public health infrastructure in the United States is vastly different than in the past, when devastating influenza pandemics hit with little warning," said Guay.



Surveillance and early detection are major steps to containing the initial outbreak of a new and potentially severe strain of influenza. Sick patients would likely go first to the medical community with flu-like symptoms, rather than go to the dentist for treatment.

"Dentists' role would be ancillary," Guay said, "but their vigilance concerning patient behaviors could still aid in the early detection of an influenza outbreak."

However, in the event of a full-scale pandemic, the medical community would not be able to control or monitor the situation on its own. Local medical resources could be overwhelmed.

"The surge capacity would be over the top, and dentists may need to play a hands-on and vital role to counteract the spread of disease and safeguard the public health," Guay said.

Infection control procedures, for example, would likely need to increase, as well as apply beyond the operatory and dental treatment into the waiting room. Dental offices may be required to close down, except for emergency treatment. Two ADA councils, according to Guay, are considering which expanded infection control practices may be appropriate for dental offices to employ in the event of a severe influenza situation.

Dentists might also be mobilized in ways similar to a response to bioterrorism, such as administering medications and immunizations or using their dental offices as temporary medical centers, Guay said.

"Which is why the ADA is urging dentists to be familiar with their state dental society's emergency response plan to keep themselves up-to-date with mass disaster training programs and, above all, to pay attention and keep informed by monitoring world health events from reliable sources.

"The ADA will help by providing information to dentists in a timely manner," Guay added.

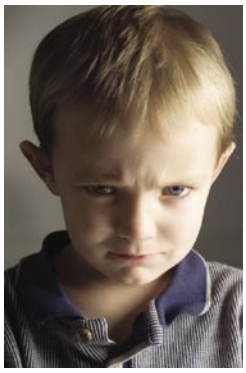
In the 20th century, there were three influenza pandemics. The most recent occurred in 1968 with the Hong Kong Flu outbreak, which resulted in close to 34,000 deaths in the United States alone. Eleven years earlier, the Asian flu claimed approximately 70,000 people. But the worst of all was the Spanish flu in 1918 that caused illnesses to an estimated 20 to 40 percent of the world's population and claimed more than 50 million individuals throughout the globe. Between September 1918 and April 1919, 675,000 Americans died from the Spanish flu.

The new report, "Influenza Pandemic," is available online in the dental topics content area of ADA.org or by calling the ADA, (800) 621-8099, Ext. 2844.

Study Shows Greater Caries Prevalence in ADHD Children

Children diagnosed with attention-deficit/hyperactivity disorder have a higher prevalence of caries compared to nondiagnosed children used as controls, reported researchers in a recent issue of *Journal of Clinical Pediatric Dentistry*.

In conducting the study, Michael Todd Grooms, DDS, MS; Martha Ann Keels, DDS, PhD; Michael Roberts, DDS, MScD; and F. Thomas McIver, DDS, MS, examined pediatric patients at Duke University Medical Center Pediatrics and Pediatric Dentistry clinics. Subjects were divided into non-ADHD and ADHD groups, with participants distributed by socioeconomic status as well.

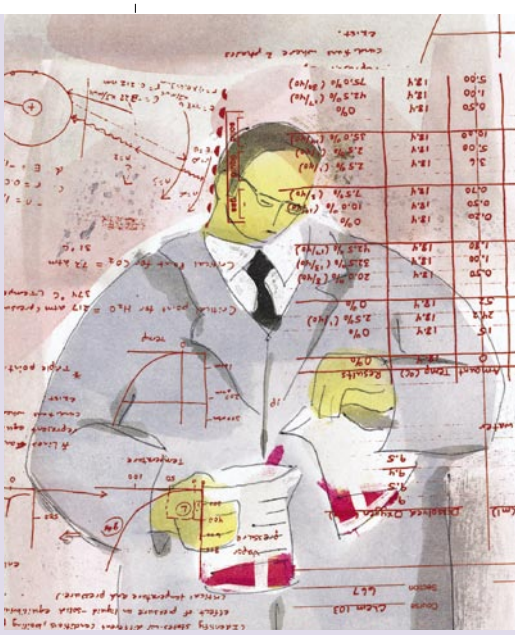


The parents/guardians of all children completed a questionnaire concerning their child's oral health including oral hygiene, diet, and fluoride use, etc.

The study was not able to identify the exact contributing factors that led to an increase in caries in ADHD children. In fact, there were no detected differences between non-ADHD and ADHD children in key preventive practices such as systemic fluoride exposure and flossing, and brushing teeth with fluoridated toothpaste. There also was no noticeable difference in diet.

The authors emphasized that dentists, knowing that ADHD children are prone to more caries incidences, may want to undertake more aggressive preventive programs for those patients.

Dentistry Taking Lead in Bioengineering



Dentists quickly are becoming the leading bioengineers of the 21st century, according to authors in an issue of *Today's FDA*, the journal of the Florida Dental Association.

In the issue, Nova Southeastern University's Peter Murray, PhD; Jonathan Coffman, PhD; and Franklin Garcia-Godoy, DDS, highlighted their work to show advances in three various areas.

Murray is working on a way to use adult stem cells to grow replacement teeth in a laboratory. The ability to create teeth that can be used to restore extracted or lost teeth has long been a goal of dentistry. Stem cell research, the authors believe, is at the forefront of achieving that goal.

Coffman is working on a bioengineering therapy. His research involves identifying adhesion proteins of oral bacteria and investigating methods to genetically change those proteins while retaining the bacteria in the mouth, thereby establishing a delicate balance of having bacteria in the oral cavity but with diminished ability to adhere to tooth surfaces.

Garcia-Godoy, meanwhile, is working to improve the stability, longevity, and tissue integration of biomaterials. He and his research team are creating new formulations of biomaterials that include medicines to try to improve recovery speed and minimize the incidence of complications.

"Bioengineering to prevent oral diseases will represent a major milestone in preventive dentistry," the authors stated, adding, "Dentistry is well-placed to take the lead position in introducing bioengineering therapies to the general population."

Dentists should know HIPAA security regulations apply only to electronic-protected health information.

New HIPAA Kit Available

An easier-to-use version of the American Dental Association's Health Insurance Portability and Accountability Act Security Kit is now available.

HIPAA security, like all HIPAA regulations, applies to dental practices that transmit electronic transactions for which the U.S. Department of Health and Human Services has established a standard either directly, through a vendor, or clearinghouse.

Electronic claims are the standard transactions most commonly used by dentists. Dentists should know HIPAA security regulations apply only to electronic-protected health information. HIPAA regulations, in contrast, apply to protected health information in all forms, whether oral, written, or electronic. The security regulations impose different requirements for safeguarding electronic-protected health information in addition to those contained in the privacy regulations. The security compliance deadline was April 20, 2005. Dentists with any questions can contact the ADA HIPAA helpline via e-mail: hipaa@ada.org or by calling (312) 440-4608.

The revised security kit, item No. J685, provides a step-by-step plan for learning and understanding the regulations, conducting a practice-specific risk assessment, and implementing changes that lead to compliance. The member price is $99.95; $149.95 for nonmembers. The kit includes:

- A CD-ROM with customizable forms, policies, and procedures in both Word and PDF formats,
- A PowerPoint employee training presentation,
- A HIPAA Security glossary or regulatory terms, and
- A list of additional HIPAA Security print and Internet sources.

The new HIPAA Security Seminar DVD, item No. X531, and video, item No. X530, features a two-hour seminar conducted by the ADA's legal and informatics experts, who break down each of the security requirements into manageable tasks. The DVD and video, which can be used for staff training, is available by calling (800) 947-4746 or through the ADA product catalog. The member price is $99.95; $149.95 for nonmembers. The DVD and video include the opportunity to earn five continuing education credits.

Dentists who already have a HIPAA Security Kit do not need the revised version to meet compliance.

Kids Like to Color Their World... and Their Teeth

A way to make children feel more involved in the placing of restorations in their primary teeth is to let them choose a specific color of filling, said Christine Schaefer, DMD, in an issue of *Dentistry Today.*

Schaefer used clinical examples to show that young patients who choose the color of their restorations were more likely to accept the idea of treatment. Additionally, Schaefer wrote, "The success of the treatment is aided even further by the dentist's explanation to the children that the fillings will continue to look good as long as the patient properly maintains them."

By utilizing colored filling materials available on the market, dentists can help kids achieve a better level of oral hygiene. Children, Schaefer said, usually are very proud of their new blue, green, or red fillings. This can make it easier for dental professionals to educate them on proper dental hygiene.

Low Complication Rate of Third-Molar Surgery in Adults

Third-molar surgery in patients 25 years of age and older has a minimal impact on the quality of life and a low incidence of complications, that's according to the results of the American Association of Oral and Maxillofacial Surgeons' Age-Related Third Molar Study, published recently in an issue of the *Journal of Oral and Maxillofacial Surgery*.

The national study of 3,700 patients and 8,300 third molars extracted by 63 oral and maxillofacial surgeons between January and December 2001 showed that "the removal of third molars in an adult patient population is a safe surgical procedure with minimal morbidity, no mortality, and no long-term negative impact on the patient's quality of life," said Richard Haug, DDS, principal author.

"This study offers the largest prospective evaluation to date of patients aged 25 years and older undergoing third-molar surgery," said Haug, who also is professor of oral and maxillofacial surgery and executive associate dean at the University of Kentucky College of Dentistry in Lexington. "It also provides oral and maxillofacial surgeons with reliable data related to preoperative risk factors and postoperative complications for this specific population. Previous studies have relied on smaller samples, all age ranges or retrospective analysis, limiting the ability for direct comparison."

Nearly one-third of patients, 31.2 percent to 34.1 percent, had only minimal inconvenience associated with their surgery, and neither missed work nor curtailed their normal activities. Intraoperative complications occurred with a frequency of less than 1 percent. None of the patients required a blood transfusion, there were no deaths, and none of the patients experienced problems with their airways after surgery, although the frequency of airway compromise was 0.5 percent.

With the exception of alveolar osteitis, which occurred with a frequency of two or three per thousand extractions, 0.2 percent and 0.3 percent, for maxillary wisdom teeth and slightly more than 1 in 10, 11.9 percent to 12.7 percent, for mandibular wisdom teeth, postoperative complications occurred with very low frequency. Numbness or tingling of the chin, lower lip, and jaw, the second-most common complication occurred with a frequency of 1.1 percent to 1.7 percent.

The study provided additional information about third-molar patients and surgery, Haug noted. For example, nearly half, 43.5 percent to 53.3 percent, of extracted teeth will be associated with some form of pathology, most frequently decay, 17.6 percent to 20.3 percent; gum disease, 11.6 percent to 17.6 percent; and infection, 6.3 percent to 16.7 percent. Most frequent third-molar extractions will involve all four third molars, 26.5 percent, followed by a combination of two molars, 0.7 percent to 5.2 percent; a single tooth, 7.2 percent to 13.2 percent; and three teeth, 2.6 percent to 2.8 percent.

"The removal of third molars in an adult patient population is a safe surgical procedure with minimal morbidity, no mortality, and no long-term negative impact on the patient's quality of life."

RICHARD HAUG, DDS

Treating Patients With Aspirated Teeth

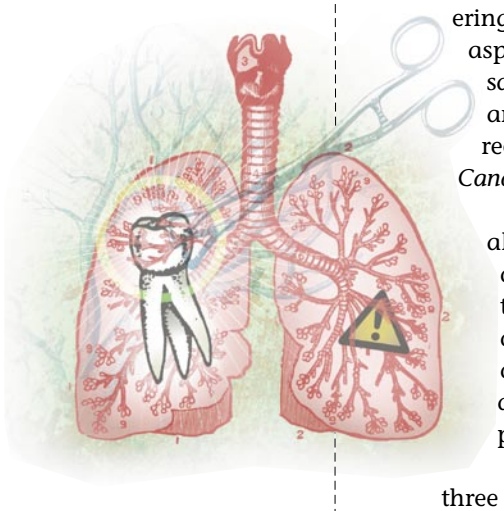


Illustration: Matt Mullin

The aspiration of deciduous teeth is surprisingly unusual; especially considering how often young children aspirate other small objects, said Jeffrey Ludemann, MD, and Juan Ospina, MD, in a recent issue of the *Journal of the Canadian Dental Association*.

Aspiration of dental materials and teeth mostly occurs as a result of blunt trauma to the face, said Ludemann and Ospina. Sometimes, an extracted tooth can be aspirated, particularly if the patient is a wiggling child.

The authors discussed the three clinical phases in bronchial foreign-body aspiration. The initial acute phase is characterized by a coughing paroxysm that lasts at least a few minutes. Next is the quiescent phase, during which time the patient is fairly asymptomatic. This is when a foreign body creates a kind bronchial check-valve, allowing air to enter the lungs, but impeding the exit of air during expiration. Lastly, approximately a week later, once bronchial mucosa becomes tightly swollen around the foreign body, a stop-valve is created. This is the complication phase.

Since 1974, there have been three cases in which diagnosis of an aspirated tooth in adult patients was delayed until the complication phase, according to the authors. One of the patients died from sepsis and respiratory arrest.

Forceps and a rigid bronchoscope generally are the best tools for removing an aspirated tooth. However, if the patient aspirated a tooth as a result of facial trauma, it may be removed using a flexible bronchoscope or, if necessary, a tracheotomy.

"When a dental patient has a coughing paroxysm and not all teeth and foreign objects can be accounted for, chest radiography is mandatory," said the authors. "Urgent medical evaluation and treatment may be necessary."

Honors

Daniel Tanita, DDS, of San Pablo, was named president of the Pacific Dental Education Foundation Board at the University of the Pacific Arthur A. Dugoni School of Dentistry. The foundation is a volunteer organization whose purpose is to promote philanthropy on the behalf of the dental school.

Upcoming Meetings

2006

April 7-9	Annual meeting of the California Society of Pediatric Dentistry, Indian Wells, Calif., www.cspa.org.
April 27-30	CDA Spring Session, Anaheim, (866) CDA-MEMBER (232-6362).
May 16-20	American Academy of Cosmetic Dentistry 22nd Annual Scientific Session, San Diego, (800) 543-9220.
May 22-27	Academy of Prosthodontics 88th Annual Scientific Session, San Francisco, www.academyprosthodontics.org.
Sept. 15-17	CDA Fall Session, San Francisco, (866) CDA-MEMBER (232-6362). Oct. 16-19 ADA Annual Session, Las Vegas, (312) 440-2500.
Dec. 3-6	International Workshop of the International Cleft Lip and Palate Foundation, Chennai, India, (91) 44-24331696.

To have an event included on this list of nonprofit association meetings, please send the information to Upcoming Meetings, *CDA Journal,* 1201 K St., 16th Floor, Sacramento, CA 95814 or fax the information to (916) 554-5962.

Salivary Diagnostics

DAVID T. WONG, DMD, DMSc

High-impact diseases, including cancer, cardiovascular, metabolic and neurological diseases, are challenging to diagnose without supplementing clinical evaluation with laboratory testing. Even with laboratory tools, definitive diagnosis often remains elusive. Three roadblocks hold back the realization of clinical diagnostics' potential: 1) definitive disease-associated protein and genetic markers; 2) easy and inexpensive sampling methods that effect minimal subject discomfort; and 3) an accurate, portable, and easy-to-use diagnostic platform. Saliva, a biofluid, that is totally noninvasive and readily available, has long been recognized to address the second roadblock.[1]

Until recently, and with the visionary investment by the National Institute of Dental and Craniofacial Research, saliva biomarker discovery and salivary diagnostic technologies are currently in development that are addressing the first and third roadblocks. It is safe to predict that the use of saliva for disease diagnostics and normal health surveillance is about five years away. This is an exciting time as we are seeing the applications of saliva diagnostics for oral diseases, which will soon be followed by systemic diseases. This will truly allow the bridging of oral health research into systemic diseases via the biofluid the filters and processes itself from the vasculature that nourishes the salivary glands into the oral cavity (**Figures 1 and 2**). Oral fluid being the "mirror of body" is a perfect medium to be explored for health and disease surveillance. The translational applications and opportunities are enormous.

A growing number of proof-of-principle examples have been established for using saliva to monitor systemic diseases and conditions. The barriers to widespread implementation of saliva diagnostics derived from

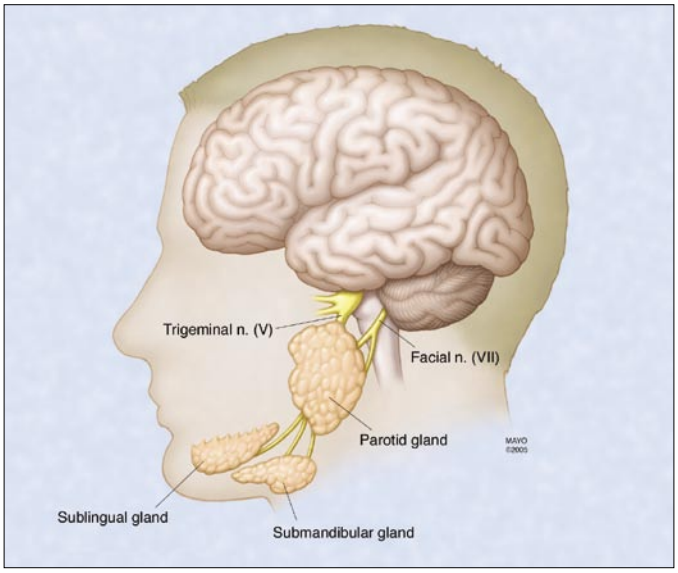


Figure 1. Anatomical locations of the three major salivary glands: parotid, submandibular, and sublingual. *By permission of Mayo Foundation for Medical Education and Research. All rights reserved. Forde MD, Koka S, et al, Systemic assessments utilizing saliva: Part 1 general considerations and current assessments.* Int J Prosthodont *19:43-52, 2006.*

Guest editor / David T. Wong, DMD, DMSc, is professor and associate dean of research in the Division of Oral Biology and Medicine at the University of California, Los Angeles, School of Dentistry. He also is director of the UCLA Dental Research Institute; holds appointment at the Division of Head and Neck Surgery/Otolaryngology, Henry Samueli School of Engineering and Applied Sciences; and is a member of the Molecular Biology Institute and Jonsson Comprehensive Cancer Center.

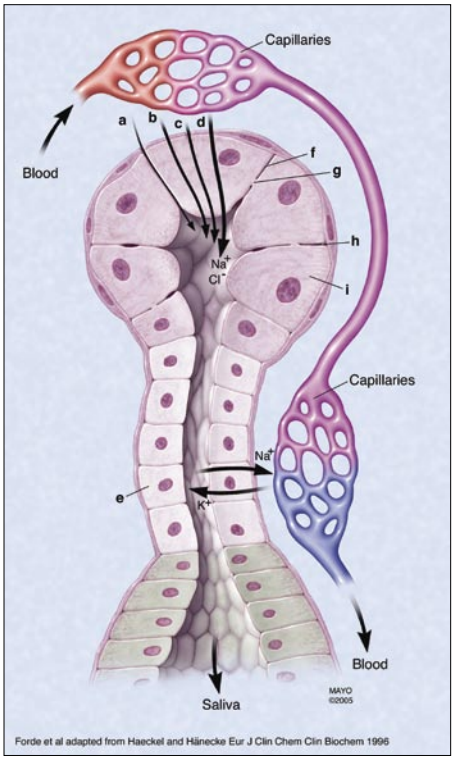


Figure 2. Mechanisms of transport of proteins and ions from serum into salivary gland ducts. *By permission of Mayo Foundation for Medical Education and Research. All rights reserved. Forde MD, Koka S, et al, Systemic assessments utilizing saliva: Part 1 general considerations and current assessments.* Int J Prosthodont *19:43-52, 2006.*

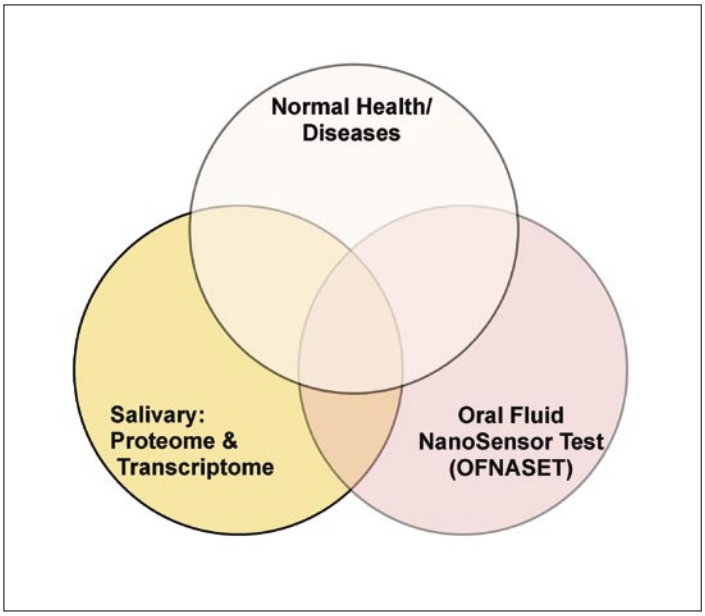


Figure 3. Disease markers manifestation in saliva and their detection by saliva diagnostic biosensors (Oral Fluid NanoSensor Test, OFNASET).

technological problems such as sensitivity, miniaturization, high throughput, automation, portability, low cost, high functionality, and speed to enable detection and measurements of multiple disease markers in saliva have largely been overcome. Techniques are emerging from a combination of miniaturization technologies, and discoveries in many different fields of biology, chemistry, physics and engineering are leading to high throughput, automated, portable, low cost, more efficient, and rapid biochemical analyses. Miniaturized diagnostic technologies will be able, with minute amounts of body fluids, to yield critical patient information reflecting health and disease status. These "lab-on-a-chip" platforms will be able to perform multiple operations in parallel in nonlaboratory settings such as the field, factory, hospital clinic, or home. It is envisioned that such technologies will allow the simultaneous assessment of multiple conditions of health and disease and provide clinicians with prevention and therapeutic strategies to meet patient needs.

Vision and Challenges

The postgenomic era provides opportunities for high-throughput approaches to genomics and proteomics. The novel technologies of miniaturization, coupled with the highly parallel detection, create the possibility of radically new ways to detect and diagnose health and disease states in an individual, even in remote or impoverished settings. These discoveries and technological advances in conjunction with the ability for disease diagnostics in a noninvasive biofluid would offer a revolutionary change in medicine.

There is a great need for convenient and accurate point-of-care disease diagnostic tools in a noninvasive manner. This is of particular relevance in the developing world where many health risks and illnesses remain poorly defined and receive inappropriate treatment. In addition, little information about the burden of disease is available to guide population health decisions.

The vision and challenge of saliva diagnostics is to discover the diagnos-

tic potential and optimize engineering technologies for this biofluid. **Figure 3** is a Venn diagram that illustrates that within the spectrum of total human health and disease states (top circle), it is envisioned that some of these states will reflect themselves diagnostically in saliva via either proteomic or genomic information (lower left circle). How much overlap will the subset remain is to be determined. The lower right circle illustrates the technology development platforms necessary to advance the point-of-care detection capability of saliva.

The challenge to making saliva diagnostics a clinical reality is to establish the scientific foundation and clinical validations necessary to position salivary diagnostics to be novel, highly accurate and feasible technologies to achieve definitive point-of-care assessment of individuals' health and disease status. Inherent in this vision is to establish the science and diagnostic targets in saliva and the development of robust, simple-to-use biosensor technologies for reliable and valid clinical applications.

In this issue of the *Journal of the California Dental Association*, we have invited a number of scientific, opinion and thought leaders in the field of saliva diagnostics to present their current research to highlight the excitement and promises of saliva diagnostics. CDA

References / 1. Mandel ID, Salivary diagnosis: more than a lick and a promise. *J Am Dent Assoc* 124(1):85-7, 1993.

A Novel Saliva Test for Caries Risk Assessment

PAUL C. DENNY, PHD; PATRICIA A. DENNY; JONA TAKASHIMA; YAN SI; MAHVASH NAVAZESH, DMD; AND JOYCE M. GALLIGAN, DDS

ABSTRACT

A new saliva test for caries risk assessment introduced in this study integrates a variety of host factors to predict for children, individual risk levels that are tooth-group specific. These various host factors correlate with caries history, DFT (decayed and filled teeth) or DFS (decayed and filled surfaces) in young adults. The test is based on the pattern of genetically determined oligosaccharides present on salivary glycoproteins. The mechanism behind the test is believed to be centered on the specific oligosaccharides that either facilitate bacterial attachment and colonization at the surface of teeth or protect against colonization by promoting agglutination and removal of free bacteria. It is the ratio of the two classes of oligosaccharides that is very strongly correlated with the numerical range of DFS or DFT observed in a young adult population.

Promoting oral health by preventing disease is a common theme in the surgeon general's report on oral health in America.[1] Caries is an infectious disease that is manifested to varying degrees in different individuals. While caries can be prevented, to be effective and lasting, prevention should be initiated before the cariogenic process begins.[2,3] Thus, if the preventive treatments are to acknowledge the variation in extent of the

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Joyce M. Galligan, DDS, is an assistant professor of Clinical Dentistry, Division of Diagnostic Sciences, University of Southern California School of Dentistry.

Acknowledgment / This project was supported by NIDCR STTR Phase I and Phase II grants, 2 R42 DE014650, "Saliva Test for Caries Risk" to Proactive Oral Solutions, Inc. Clinical studies were performed under subcontract to the University of Southern California School of Dentistry. CARE Test is the registered trademark of Proactive Oral Solutions, Inc. The commercial test is in midphase development and will soon be market-ready.

disease between individuals while minimizing irreversible, invasive treatment, a prediction of the anticipated degree of the disease is needed. This is often designated as a risk level.

One of the earliest acknowledgements that different risk levels exist was the assignment of individuals into "caries-prone" and "caries-resistant" groups.[4] This system is based on the significant positive correlation between past caries history and the number of future lesions, but is inadequate in at least two areas.[5,6] First, because the number of caries in the population is a continuum, approximately 50 percent of patients are unassignable to either group. Thus, if a middle-risk level is formed, regardless of the criteria, it contains individuals who are not statistically different from those within either of the extreme-risk groups.[7] The other shortcoming is that the prediction is based on existing caries for which prevention is not possible.

Currently, the use of a questionnaire, in combination with clinical observation, is a popular approach for caries risk assessment.[8,9] The questionnaires play an important role by revealing medical conditions and/or health and eating habits that are known to increase the risk of developing caries. The remainder of the questionnaire often addresses many of the other factors that contribute to the multifactorial genesis of caries. This approach leads to a generic assessment of risk but, at an evidence-based level thus far, does not provide personalized prognoses that exceed a combined score of 180 percent for sensitivity and specificity.[6] Most often, the result is given as one of three risk levels based on the overall judgment of the care provider after consideration of the results of the questionnaire and clinical findings, which may include oral microbial assessment and saliva flow rate.[7,8] In this system of risk assessment, though various factors are sampled, there is no clear prediction of specific consequences and thereby fewer opportunities for targeted preventive treatments. However, in conjunction with the saliva-based test that does identify specific groups of teeth at risk, the authors believe that the prospect of raising children who are caries-free, regardless of risk level, is possible with focused prevention.

The technology used by the caries-risk test, CARE Test, discussed here, samples host factors that are thought to be involved either in bacterial attachment to the tooth surface or with agglutination of free-swimming bacteria. The host factors are specific oligosaccharides (sugar chains) attached to the glycoproteins in saliva. These oligosaccharides are in the same class of compounds as blood-type determinants, and thus are genetically determined. The mechanism of interaction between a particular oligosaccharide and an oral bacterium occurs by way of surface receptors called lectins, which are specific for a particular sugar and its linkages within the oligosaccharide. This study showed that certain oligosaccharides are positively correlated with caries history, as defined by DFS or DFT, and others are negatively correlated.

When the quantities of specified oligosaccharides in an individual's saliva are integrated, the test yields a single output value that is highly correlated with caries history. The test can be further modified to yield risk levels that reflect the groups of teeth with active and restored carious lesions. Using a test that has been calibrated in young adults and then applied to children, a prediction of their future pattern of caries infection is generated. Each risk level has a treatment plan that is targeted to the teeth at risk.

Dot Blot for Quantitation of α-2,6-Linked Sialic Acid in Oligosaccharides Using the Lectin SNA from *Sambucus Nigra*

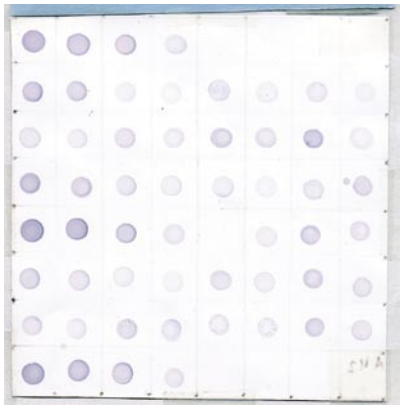


Figure 1. Representative assay of lectin affinity to spots containing the equivalent of 0.2 or 0.1 µl of whole saliva from different individuals. Each spot is quantitated relative to a standard curve (four dots, top line, left) and is based on a combination of area and average intensity.

Test Methods and Applications

Subjects

The young adults, who were selected to be the reference group for the caries risk assessment process, were volunteer students from the dental and dental hygiene programs at the University of Southern California School of Dentistry. They ranged from 24 to 34 years old. This age range represented a stable statistical endpoint for first-time caries acquisition as judged by a lack of significant correlation of their ages with caries history. Inclusion of younger subjects elevated age to a significant variable in the regression analyses. The subject group included a variety of races and ethnicities. The backgrounds of these subjects suggested the majority were from family environments where mainstream oral health care standards were practiced throughout their formative years. Thus, the endpoint of the test, which had been calibrated with these subjects, is not intended to be maximally predictive in individuals lacking good oral hygiene or nutritional habits known to be associated with an increased risk for rampant caries.

Children 7 to 10 years old were

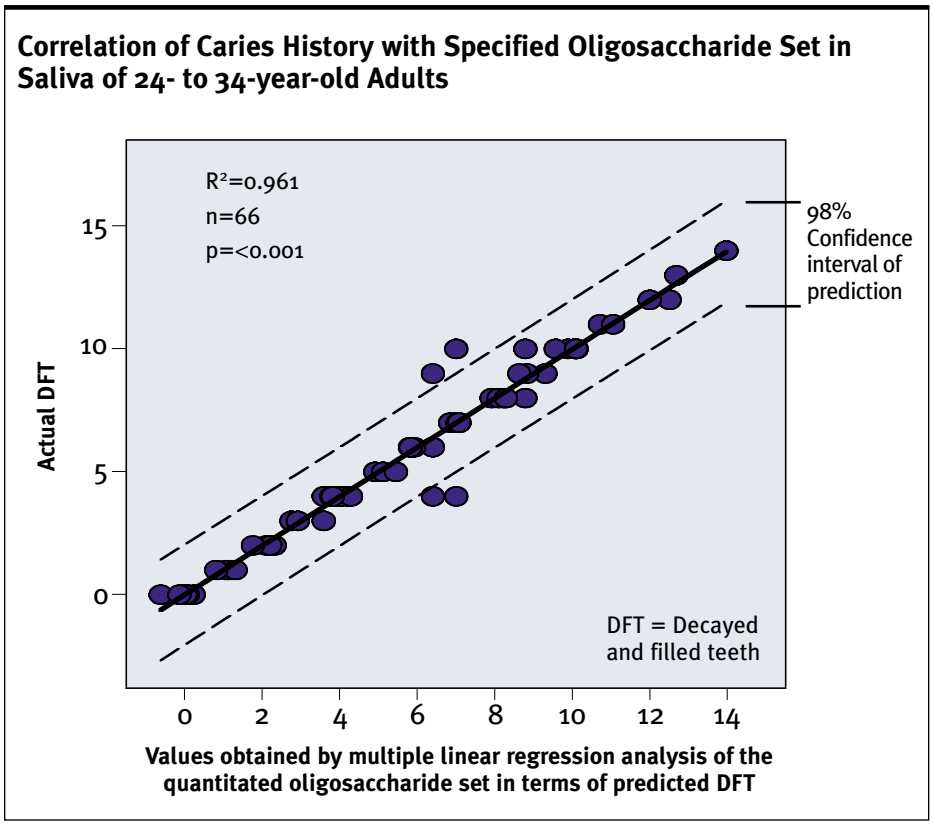


Figure 2.

selected as the target group because they represented an endpoint for the caries history of their deciduous teeth (dfs-decayed and filled surfaces) and also an early stage of caries history for their permanent teeth (DFT). The children included in this report were equally split between Asians and Hispanics. The study is continually expanding the variety of races and ethnicities that are represented in the target group. The goal is to "universalize" the applicability of the test as much as possible. Targeting groups of children that deviate broadly from the reference subject norm may require a different calibration strategy.

Saliva Collection

Resting whole saliva was collected by drooling after a fast of at least one hour.[10] All dental examinations were visual and followed the saliva collection.

Oligosaccharide Quantitation

The different species of oligosaccharides were quantitated by dot blot technology (**Figure 1**). Dried spots containing whole saliva were probed with commercially available lectins, which have affinities for different oligosaccharides. The amount of lectin binding was proportional to the amount of a particular oligosaccharide present in the spot of saliva. The bound lectin was then visualized and quantitated relative to the blood glycoprotein, glycophorin. The range of variation between subjects for different oligosaccharides within the reference group was from as little as three-fold to more than 5200-fold. The combination of lectins used in the test is proprietary.

Mathematical Procedures

Linear regression has been reported to be one of best approaches for evaluating correlation of risk factors.[5] All results presented here have been achieved by multiple linear regression analyses, though the final version of the test may involve a different mathematical approach that achieves the same results. Where appropriate, statistical measures, such as significance and coefficient of determination (R^2), were reported. SigmaStat (SPSS, Inc.) was the primary statistical program used for this study.

Test Development

The first indication of a relationship for caries history and host factors was a strong correlation between the concentration of MUC7 and MUC5B mucins in saliva of young adults and their DFT.[11] Subsequent studies led to narrowing the focus to the oligosaccharides of the mucins and later, simply to the quantity of specific oligosaccharides in whole saliva. The relationship between the mathematical integration of the oligosaccharide quantities and caries history of young adults is shown in **Figure 2**. The vertical width of the 98 percent confidence interval is equivalent to an error bar at any point on the regression line. A similar relationship was found between concentration of specific oligosaccharides and caries history in the deciduous teeth (dfs/t) in 7- to 10-year-old children (**Figure 3**). Here, the examination metric has been changed to a ratio to accommodate the number of remaining deciduous teeth (t) in each child. The correlations for both children and young adults are highly significant.

Test Applications

Beyond its use as an analytical tool, a goal of regression analysis is to be able to predict values for the dependent variable from the independent variables. The model for predicting caries risk lev-

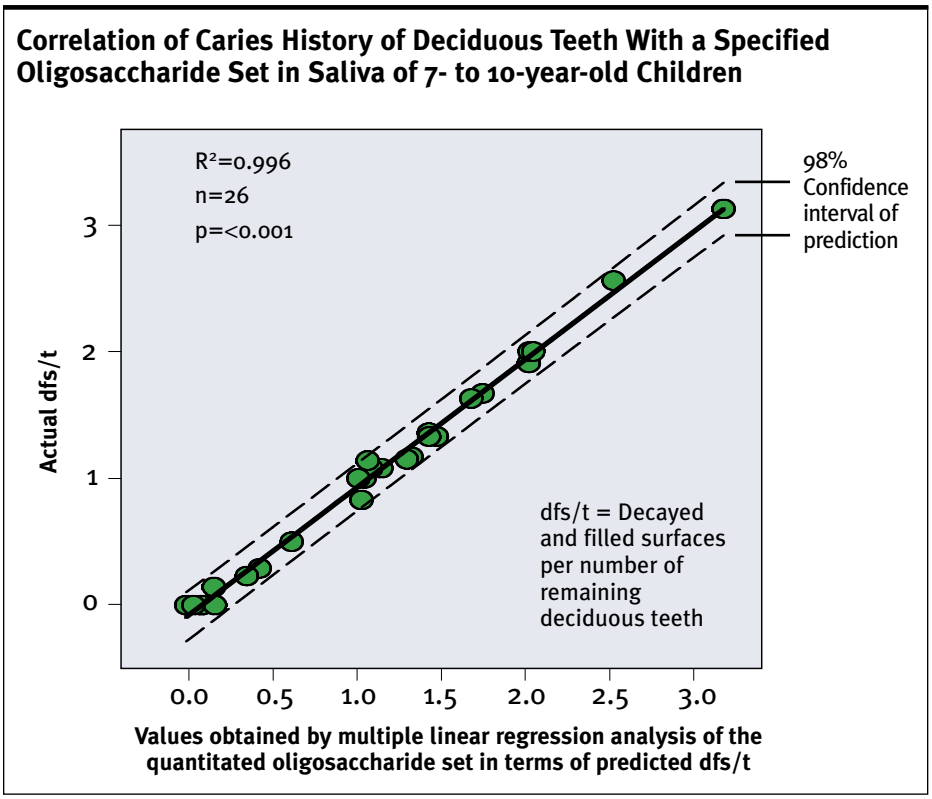


Figure 3.

els in children, which are translatable into effective preventive treatment regimens, is presented in **Figures 4a** and **4b**. The first step reorganizes the adult's caries history (DFT) into groups of teeth for which a targeted treatment would have prevented the included caries. Then the individual oligosaccharide datasets that relate to the risk groups were identified in the adult reference group, and the regression analysis was begun. As the analysis neared completion, selected children, at intermediate stages of their caries history, were included with the adult group to "train" the final regression analysis to apply to both adults and children. The performance of this regression equation, which relates the quantitated oligosaccharide data from adults to the risk levels, is shown in **Figure 4a**.

The saliva samples from the children were quantitated for the same oligosaccharides that defined the regression equation for the adults, and the regression equation was then applied to the dataset from the children. The children fell into the same risk categories as those established for the adults (**Figure 4b**). In this case, the prediction is a projection of the caries history that each child will have when they reach 24 years old, unless preventive measures are begun. Depending on the group of teeth at risk, the suggested preventions range from sealing all 16 posterior teeth with fluoride applications on the remaining teeth for those in the highest risk group to no additional treatment for those in the lowest risk level. Treatments for the intermediate risk levels focus on the specific teeth at risk.

The real-time correlation of oligosaccharide patterns and caries history suggests that the test has the capability to serve as a screening diagnostic. In essence, the caries history component of the correlation can be mathematically re-programmed to yield the desired distribution. An example of this is shown in **Figure 5**, where the outcome is calibrated to separate children who have no caries from those who have caries in either or both deciduous and permanent teeth. If age of the individuals is included in the regression analysis, caries history (DFT) can be forecast in groups that include both children and adults at a similar level of accuracy to that achieved for either group alone.

Discussion and Conclusions

One of the fundamental tenants of the oligosaccharide-based caries risk assessment test is that a key determinant of caries history is related to genetics. While the accuracy of the test speaks for itself, what is the corroborating evidence of this genetic component? First, there is no doubt that the oligosaccharide patterns on salivary glycoproteins are of genetic origin.[12] In saliva, one of the main carriers of the oligosaccharide determinants for the ABH and Lewis blood types is MG1, the high molecular weight mucin.[13] The evidence for a genetic component in caries history comes largely from studies that compare caries patterns between monozygotic and dizygotic twins. Advantages of this approach are that very complex genetic interactions can still be evaluated and that environmental influences, which often confound familial studies, are reduced in importance. Virtually all of the studies performed in this manner have shown a strong, statistically significant genetic component for determining caries history.[14,15]

There are numerous reports of the interaction of glycoproteins in saliva with oral bacteria.[16,17] These interactions include those with glycoproteins that form the saliva-based, pellicle coating of the teeth, which is thought to

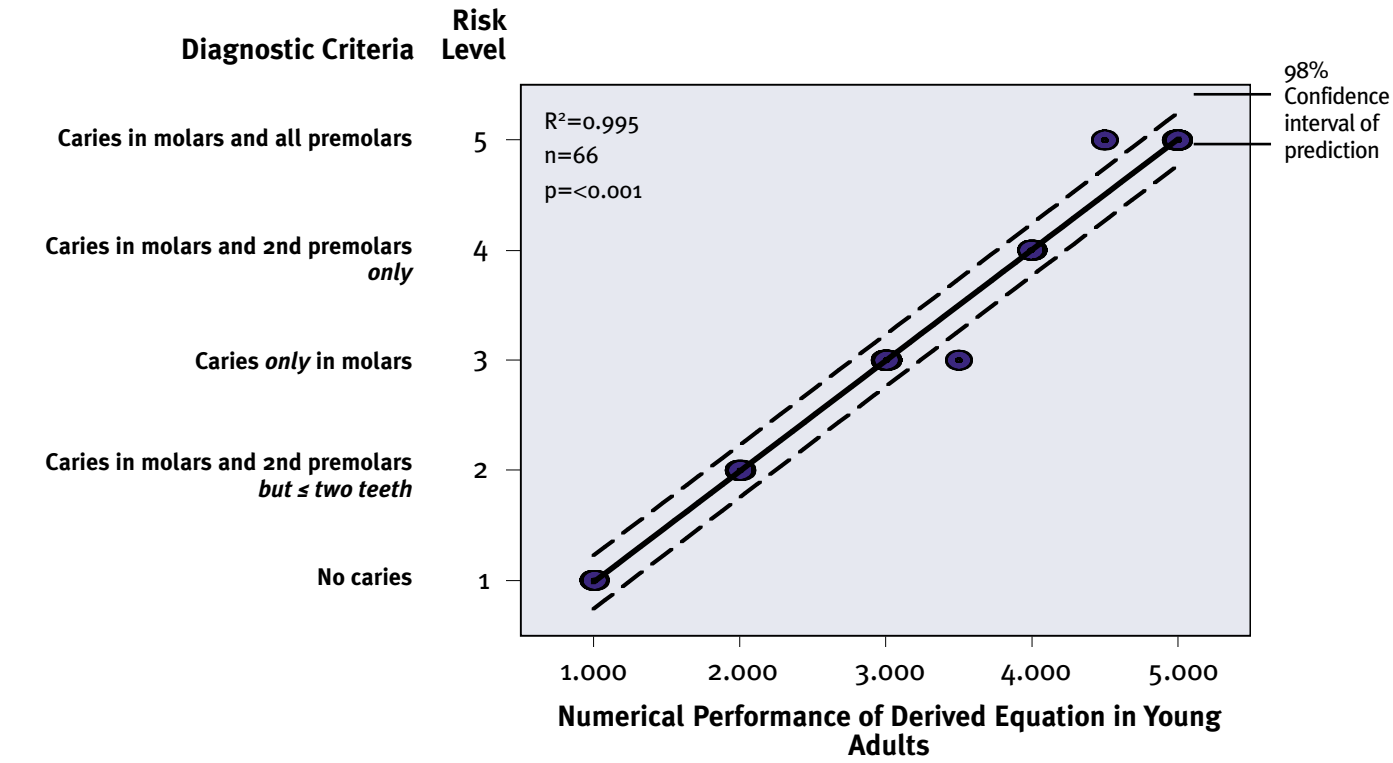
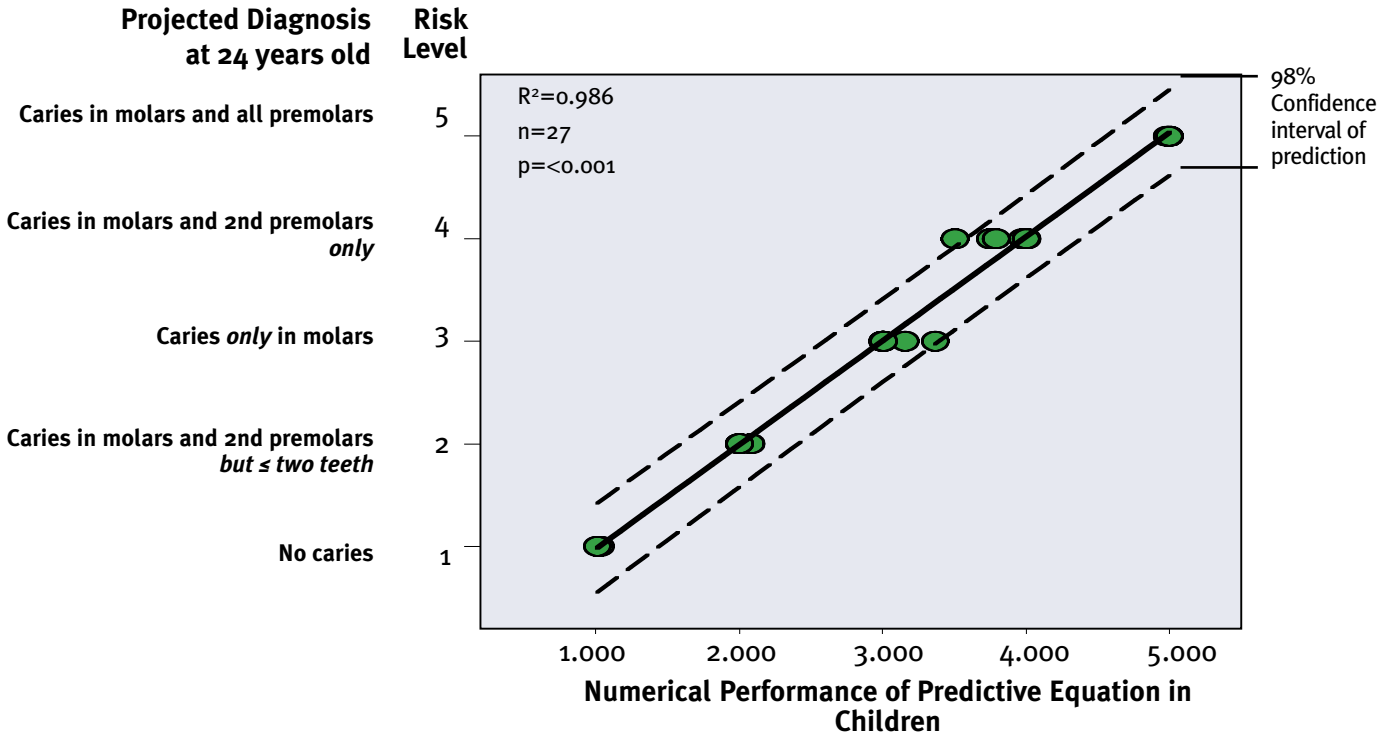


Figure 4a.

provide specific attachment sites for oral bacteria. Reports also include interactions with non-pellicle forming glycoproteins that can agglutinate oral bacteria and prevent attachment. In this class of glycoproteins, the MUC7 mucin has binding sites for selected oral bacteria and host neutrophils, providing a theoretical mechanism for enhancing phagocytosis.[18] The properties of bacterial binding to pellicle proteins and bacterial agglutination by non-pellicle proteins were found to vary between individuals. In the case of the former, there is a positive correlation of cariogenic bacteria binding with caries history, and for bacterial agglutination strength, a negative correlation.[19,20] Some of the variability between individuals' saliva in bacterial agglutination strength appears to be due directly to differences in blood type.[21]

The role of pathogen surface lectins in infectious diseases has been broadly described and is generally considered to be a common phenomenon.[22] Indeed, a bacterial lectin that binds specifically to the sialic acid end of one of the most common trisaccharides on MUC7 mucin has been isolated from oral bacteria.[23] Following desialylation of the trisaccharide, the bacteria no longer binds and are not agglutinated. The level of binding of the lectin PNA (peanut agglutinin) has shown a correlation with caries-resistant and caries-susceptible children, suggesting that elevated levels of galactose β1,3 galactosamine on salivary glycoproteins may help protect against caries.[4] The present study has found several other lectins that alone suggest either significant negative or positive correlations with caries history. However, none of the lectin affinities individually provide the strength of correlation that can accurately predict individual caries histories or risk levels. The failure of single lectin affinities to provide individually accurate forecasts can be attributed to the variety of genes that

Model for Prediction of Risk Levels in Children — Part 2: Application of the Predictive Equation to the Saliva of Children



Step 3: Quantify the affinities of the previously identified battery of lectins in the saliva of 7- to 10-year-old children.

Step 4: Apply the predictive equation.

Figure 4b.

lead to the complex structure of individual saliva glycoprotein oligosaccharides, which may contain more than 15 sugars.[12,13] In contrast, the caries risk assessment test described in this report measures a variety of lectin-sugar combinations to identify and quantitate signature elements of the oligosaccharide chains that are specifically related to caries history. CDA

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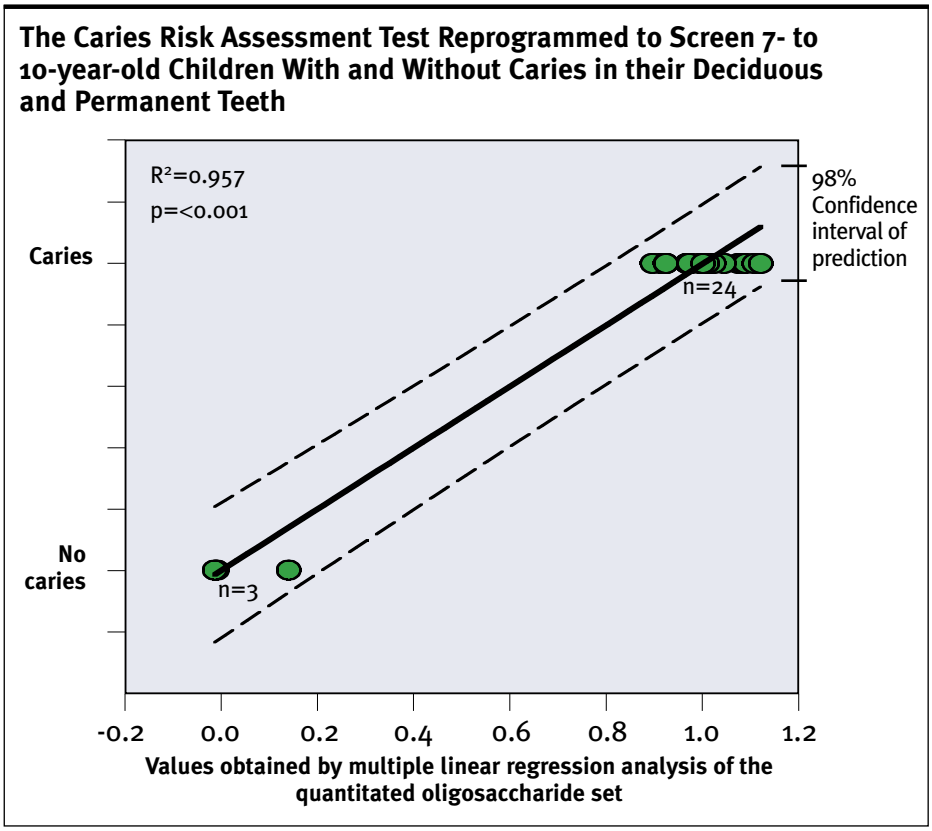

Figure 5.

Salivary Diagnostics for Oral Cancer

DAVID T. WONG, DMD, DMSc

ABSTRACT

Oral cancers annually strike 38,000 individuals in the United States and hundreds of thousands of others around the globe. Despite treatment advances, the disease's overall five-year survival rate has not improved in the past three decades and remains among the worst of all cancers. One factor behind oral cancer's high mortality is the challenge detecting it at its early stages. The use of saliva for the detection of oral cancer has been a historical goal that has yet to come to fruition. This review highlights translational research efforts in alignment with initiatives sparked by the National Institute of Dental and Craniofacial Research toward bringing saliva diagnostics to fruition and, in particular, for saliva-based oral cancer detection.

The ability to monitor health status, disease onset and progression, and treatment outcome through noninvasive means is a most desirable goal in the health care promotion and delivery. There are three prerequisites to materialize this goal: specific biomarkers associated with a health or disease state; a noninvasive approach to detect and monitor the biomarkers; and the technologies to discriminate the biomarkers. The author presents a roadmap to achieve these goals through the use of oral fluids (saliva) as the diagnostic medium to scrutinize the health and/or disease status of individuals. This is an ideal opportunity to bridge state of the art micro-/nano-electromechanical system (MEMS/NEMS) sensors to oral fluid for diagnostic applications. With oral fluid being the "mirror of body,"

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it is a perfect medium to be explored for health and disease surveillance. The translational applications and opportunities are enormous.

A growing number of proof-of-principle examples have been established for using saliva to monitor systemic diseases and conditions. The barriers to widespread implementation of saliva diagnostics, derived from technological problems such as sensitivity, miniaturization, high throughput, automation, portability, low cost, high functionality, and speed to enable detection and measurements of multiple disease markers in saliva, have largely been overcome. Techniques emerging from a combination of miniaturization technologies and discoveries in many different fields of biology, chemistry, physics, and engineering are leading to high throughput, automated, portable, low cost, more efficient, and rapid biochemical analyses. Miniaturized diagnostic technologies will be able, with minute amounts of body fluids, to yield critical patient information reflecting health and disease status. These "lab-on-a-chip" platforms will be able to perform multiple operations in parallel in nonlaboratory settings such as the field, factory, hospital clinic, or home. It is envisioned that such technologies will allow the simultaneous assessment of multiple conditions of health and disease and provide clinicians with prevention and therapeutic strategies to meet patient needs.

Oral Cancer

Oral cancers are the sixth-most common cancer in the United States, affecting 38,000 Americans yearly and killing 7,200. Worldwide, they annually affect an estimated 350,000 individuals. More than 90 percent of these cancers are squamous cell carcinoma. Despite treatment advances that have resulted in reductions in patient morbidity, the overall five-year survival rate for oral squamous cell carcinoma remains among the worst of all cancer death rates, approximately 30 percent to 40 percent for the past few decades, considerably lower than those for colorectal, cervix, and breast origin.[1,2] One patient dies from oral cancer every hour in the United States. This high morbidity rate can be attributed to factors including nonresponsiveness to chemotherapy and radiation therapy, late presentation of lesions, and a lack of satisfactory biological markers for early lesion detection.[3] According to The Oral Cancer Foundation, oral cancer is particularly dangerous because it has a high risk of developing second, primary tumors. Patients who survive a first encounter with the disease have up to a 20 times higher risk of developing a second cancer.

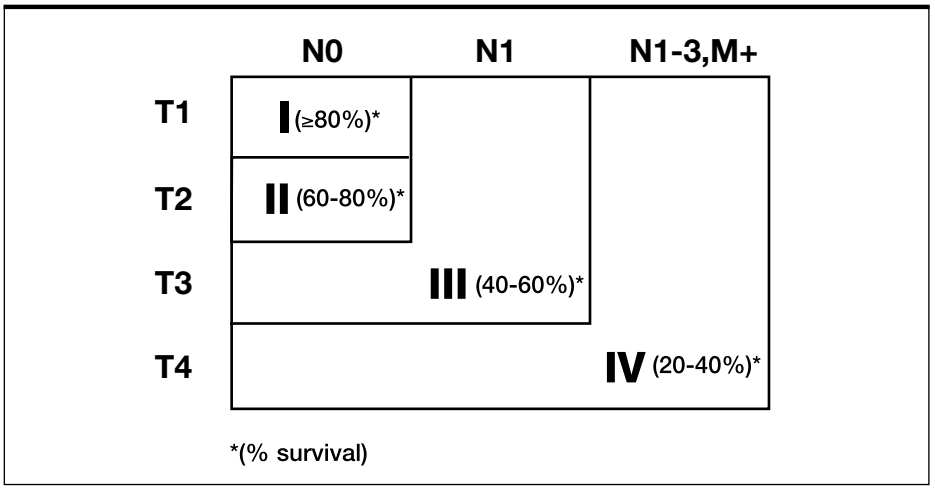

Figure 1. Clinical stage and survival rates of oral cancer. The survival rates have not changed in the past 30 years.

Current Oral Cancer Diagnostic/ Screening Approaches

The most definitive procedure for oral cancer diagnosis is a scalpel biopsy, followed by the careful histopathological evaluation by a qualified pathologist. For this to be an effective procedure, it requires three consecutive events: a visit to the dentist/physician's office, the biopsy by the licensed health care provider, and a pathologist's evaluation. When effectively administered and reimbursed, as is in the Scandinavian countries, this can lead to early detection of oral cancer lesions that otherwise would have progressed to later stage cancer, which carries a worse prognosis. **Figure 1** illustrates the prognostic difference of an oral cancer lesion as it advances down the slide of later stage lesion. Detection of an oral cancer at Stage I will carry a prognosis of 80 percent survival, while the same lesion, when it progresses to Stage III, will carry a 20 percent survival. This is a dramatic difference that will affect not only the quality of life for the patient, but a significant savings on the health care costs on the medical treatments of a Stage I versus Stage III oral cancer patient.

With this in mind, scientists have been searching for alternative approaches to biopsy, with the hope to come up with "the Pap smear" test for oral cancer detection, which has significantly improved the mortality of cervical cancer. Since most oral cancers arise as asymptomatic small lesions, only when the clinician or patient notes abnormal tissues do formal diagnosis procedures begin.[4] Microscopic investigation of the progressive cancer is often conducted too late for successful intervention.[5] It is also impractical to use

imaging techniques for cancer screening, since they are time-consuming and expensive. These techniques are typically used for confirmation because of their insensitivity for small lesions.[6] Studies have demonstrated that a good, positive predictive value can be achieved by oral cancer tissue staining with Toluidine blue.[7,8] However, extensive experience is required in applying this technique and in interpreting its results. Exfoliative cytology may be a less invasive method for oral cancer detection, but exfoliated cancer cells tend to correlate with tumor burden, with lower rates of detection seen in those with minimal or early disease.[9] A number of molecular-based diagnostic markers have been used to detect the presence of oral squamous cell carcinoma with varying degrees of sensitivity and specificity. DNA markers include TP53, microsatellite instability, and the presence of HPV and EBV genomic sequences.[10-13] Cytokeratins have been used for RNA diagnostics while SCC, CD44, CYFRA and telomerase have been used for protein markers.[14,15] None of these markers, however, universally identifies oral squamous cell carcinoma. Microsatellite markers are the most promising amongst these, where at least one of a panel of 23 markers can detect the presence of an oral cancer cell in the saliva of 79 percent of oral cancer patients.[16-19] Microsatellite instability analysis, however, is not particularly sensitive and requires a large amount of cancer cell DNA, about one cancer cell among 200 normal cells. Further, it is difficult to perform on a large number of clinical samples because many markers are necessary for testing.[16]

Recently, a number of clinical diagnostic aids for oral cancer detection have emerged. They include the OralCDx, ViziLite and Toluidine blue. The OralCDx is a brush biopsy, a non-invasive chairside procedure to determine if an oral lesion is benign or potentially harmful. Precancerous and early stage oral cancerous lesions can be determined. All suspicious lesions with abnormal cytology will be required for a biopsy follow-up. The ViziLite visual examine system is based on differential density of the nuclear content and mitochondrial matrix of abnormal cells is typically greater than normal cells. The increased molecular density is believed to reveal the increased proliferative rate and metabolic activity of precancerous cells. The ViziLite exam enhances the examiner's ability to see the difference in the nuclear/cytoplasmic ratio of dysplastic cells. After rinsing with a dilute acetic acid solution, the dense nucleus of abnormal squamous epithelium tissue will appear white when viewed under a diffuse low-energy wavelength light. Normal epithelium will absorb the light and appear dark. ViziLite can identify an abnormality, but a definitive diagnosis only can be made by biopsy.

DESPITE TREATMENT ADVANCES THAT HAVE RESULTED IN REDUCTIONS IN PATIENT MORBIDITY, THE OVERALL FIVE-YEAR SURVIVAL RATE FOR ORAL SQUAMOUS CELL CARCINOMA REMAINS AMONG THE WORST OF ALL CANCER DEATH RATES.

History of Using Saliva for Oral Cancer Molecular Detection

The use of saliva for oral cancer screening or diagnostics is still in its infancy. Its use began by a report of a small study in Taiwan by Liao et al. in 2000 claiming that exon 4 codon 63 of the p53 gene is mutated in salivary DNA from 5/8 (62.5 percent) of oral cancer patients.[20] Five of 27 control subjects (18.5 percent) had similar mutations in their p53 gene. El-Naggar et al. in 2001 demonstrated genetic heterogeneity in saliva from patients with oral squamous carcinomas and suggested the use of epithelial cells in saliva from patients with head and neck squamous tumorigenesis for genetic analysis.[21] More recently, Jiang et al. reported the increase of mitochondrial DNA content in the saliva of head and neck cancer patients.[22] Another report from the same group reported that quantitative analysis of HPV 16 DNA in salivary rinses allows for detection of HPV-related head and neck cancer.[23] However, the authors cautioned that specific limitations exist that prevent the application of this as a screening technique for a broad population.

The UCLA Approach to Saliva Diagnostic for Oral Cancer

The laboratory at the University of California, Los Angeles, utilizes research platforms toward the global identification of disease signatures in saliva. The premise of the author's approach is that since serum contents, such as disease biomarkers, will be largely present in saliva, oral fluid is a logical source to harness disease biomarkers. The lab employed both a proteome-wide as well as a genome-wide approach toward identification of disease biomarkers and signatures.

Human salivary proteome as targets for human disease diagnostics

UCLA is one of the three NIDCR-funded groups to comprehensively decipher human salivary pathogenesis. Three-hundred and nine distinct proteins in human whole saliva using 2-DGE/MS and

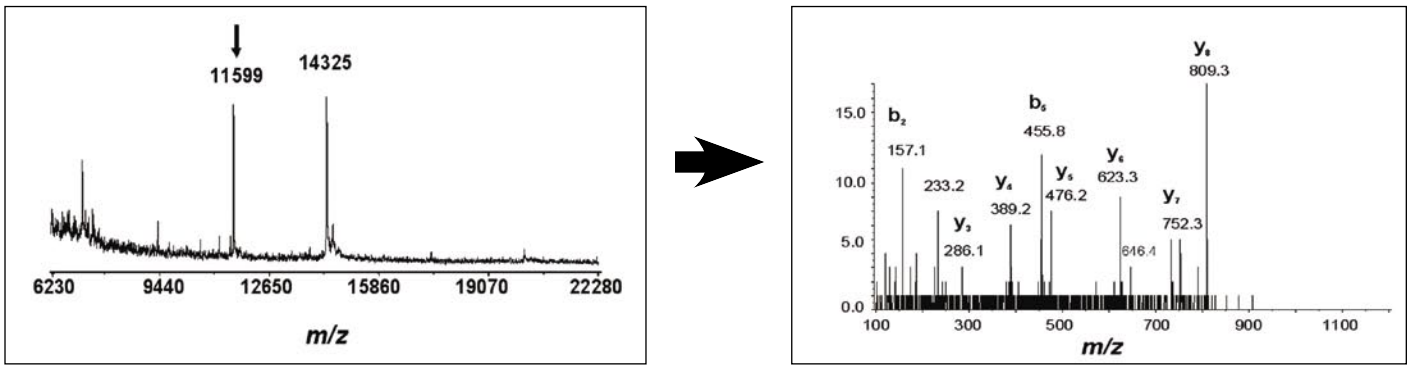

Figure 2. Identification of a potential saliva protein marker for oral cancer. Oral fluid sample was separated by LC (C4 column) and fractions collected. Panel A depicts the MALDI-MS spectrum of a LC fraction containing the protein of ~11600 Da. This fraction was digested for LC-MS/MS analysis. Panel B depicts the MS/MS spectrum of a tryptic peptide, VGEFSGANK, originated from thioredoxin.

"shotgun" proteomics have been identified. This work was recently published in *Proteomics* and highlighted in *Journal of Proteome Research* in 2005.[24] Using a similar approach, comparative proteome analysis of submandibular (SM) and sublingual (SL) saliva were conducted.[25]

To date, two salivary proteins, IL8 and thioredoxin, which can discriminate saliva of oral cancer from control subjects, have been discovered. IL8 was discovered through previous tissue-based expression profiling effort.[26] IL8 is significantly elevated in saliva of oral cancer patients and is highly discriminatory of detecting oral cancer in saliva (n=64) with an receiver operator characteristic value of 0.95, sensitivity 86 percent, and specificity 97 percent at cutoff of 600 pg/ml.[26,27] Of interest is that both IL8 protein and RNA are concordantly increased.[27] The concentration of IL8 protein in saliva of oral cancer patient and control subjects are 750± 236 pg/mL and 250± 130 pg/mL, respectively. Similarly, for salivary IL8 mRNA concentration, oral cancer patients are significantly higher than in control subjects. Due to the frequent inflammation association of this cytokine, it has been further demonstrated that the oral cancer elevation of salivary IL8 mRNA and protein is significantly higher than in advanced periodontitis patients.

These results allow for the conclusion that while severe inflammation in the oral cavity, as in advanced periodontitis patients, does elevate salivary IL8 protein and mRNA levels, it is not significant. Salivary IL8 protein and mRNA levels in oral cancer patients are elevated significantly above those of control patients as well as advanced periodontitis patients, supporting the use of salivary IL8 as a biomarker for oral cancer detection.[28]

Thioredoxin was discovered as salivary oral cancer biomarkers by a proteomic approach using MALDI-TOF. It has been established as an integrated methodology to sequence candidate protein/peptide biomarkers. Using MALDI-MS profiling of saliva proteins, it was identified that a ~11600 Da protein was present at a significantly higher level in oral cancer saliva than matched control subjects ($p<0.01$). To identify this candidate biomarker, an oral cancer saliva sample was fractionated by reverse-phase LC (C4 column) followed by MALDI-MS of the LC fraction containing the candidate biomarker of ~11600 Da (**Figure 2a**). This fraction was subsequently digested by trypsin for LC-MS/MS analysis. **Figure 2b** shows the tandem MS spectrum of a double-charged tryptic peptide, VGEFSGANK, originated from thioredoxin. Mascot database searching indicated that, totally, 4 peptides were matched to this protein, with a sequence coverage of 31 percent. These results suggested that saliva thioredoxin is a validated biomarker for oral cancer detection.[29]

Human salivary transcriptome as targets for cancer diagnostics

The UCLA research group recently found that there are approximately 3,000 human mRNAs in normal subjects' cell-free saliva.[30] Further, there is a core signature of 185 mRNAs present in all normal subjects, which provides the rationale for the use the salivary transcriptome for disease diagnostics. The discovery that a large panel of human RNA can be reliably detected in saliva gives rise to the potential of this novel clinical approach. The diagnostic value of this approach was evaluated by using oral squamous cell carcinoma as the proof-of-principle disease and found that of the ~3,000 mRNAs, seven salivary RNAs were consistently elevated in saliva from oral cancer patients. Of these, four in combination (OAZ-1, SAT, IL8 and IL1-β), have the ability to discriminate saliva from oral cancer patients from that of control subjects, with an receiver operator characteristic value of 0.95, a specificity of 91 percent, and a sensitivity

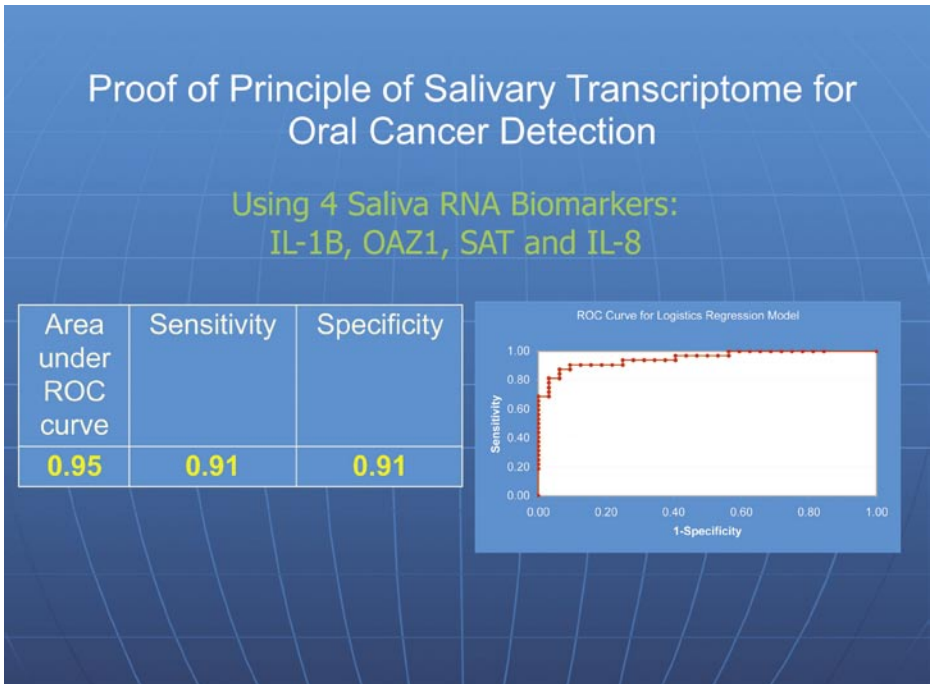

Figure 3. Receiver operator characteristic curve analysis for the predictive power of combined salivary mRNA biomarkers. The final logistic model included four salivary mRNA biomarkers, IL1B, OAZ1, SAT and IL-8. Using a cut-off probability of 50 percent, a sensitivity of 91 percent and specificity of 91 percent by receiver operator characteristic was obtained. The calculated area under the receiver operator characteristic curve was 0.95.

of 91 percent[31] (**Figure 3**). While the initial study was done on 64 subjects, three additional independent clinical detection studies with 272 subjects have since been carried out, and found that the seven saliva mRNA biomarkers behaved very consistently with an overall accuracy rate of 85 percent.[30] The discovery of RNA biomarkers in saliva that can have oral cancer discriminatory ability is a novel finding. This is now being explored of its translational potential and value.

One often wonders which bodily fluids (blood, saliva, urine, cerebral spinal fluid) are more clinically diagnostic for a specific disease entity. The author recently made that comparison for oral cancer. The same patients identified with a saliva RNA signature for oral cancer detection were examined for serum RNA signatures for oral cancer detection. Similar to saliva, four RNA biomarkers collectively can mark saliva of individuals with oral cancer with an receiver operator characteristic value of 0.88.[32] While this is very good, the salivary RNA biomarkers have an receiver operator characteristic value of 0.95.[31] Thus for oral cancer detection, saliva RNA biomarkers have a slight edge over serum RNA biomarkers.

Saliva diagnostics for other high-impact systemic diseases

Saliva has been examined for the detection of a number of systemic diseases ranging from infectious diseases, including HIV to Alzheimer's.[33-37] The author's laboratory has begun to explore a number of efforts to identify high-impact systemic diseases and explore their diagnostic signatures in saliva. Breast cancer is the first systemic disease to be explored of the presence of proteomic and genomic signatures in saliva of breast cancer patients. It should be noted that Charlie Streckfus reported that Her-2 and CA15-3 levels are elevated in cancer versus control subjects' saliva.[38,39] It is also the intent of the UCLA group to carry out rigorous proteome- and genome-wide discovery efforts to identify and validate salivary proteomic and genomic biomarkers in breast cancer patients. The research will follow guidelines from the NCI Early Disease Research Network for biomarker validation, similar to the ongoing oral cancer biomarker validation.[40]

Future Perspectives

While it is clear there is a national agenda to turn saliva diagnostics into a clinical and commercial reality, much work needs to be done before this vision can be realized. There remains the need to identify definitive disease-associated salivary biomarkers (proteins and genetic) that can be use in conjunction with the technology platforms for saliva diagnostics. The UCLA group is set to develop and validate the Oral Fluid NanoSensor Test, OFNASET, as a point-of-care chairside, portable and multiplexible device to be used for saliva diagnostics. In addition to the research infrastructure, the UCLA School of Dentistry can fully harness and validate proteomic and genomic biomarkers in saliva for human disease diagnostics. Collectively, technology platform advancement and the identification and validation of robust and discriminatory suites of salivary biomarkers for disease diagnostics represent the necessary marriage to propel saliva diagnostics into a clinical and commercial reality. At the same time, we are building the scientific foundation toward the use of saliva as a diagnostic fluid.[41] Questions have arisen, such as where do salivary biomarkers, proteins, and RNA come from? Control mechanism of salivary RNA turnover and the fate of these RNA are currently being addressed.

This is a perfect example of translational research in reverse, based on a highly relevant clinical observation that saliva contains proteomic and genomic biomarkers for oral cancer detection, and building a scientific foundation toward the mechanistic background so as to allow us to better exploit the full clinical potential of saliva diagnostics. CDA

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Oral Fluid Testing: New Technology Enables Drug Testing Without Embarrassment

EDWARD J. CONE, PHD

ABSTRACT

Drug abuse is a widespread problem across society, and its negative impact on performance and safety in the workplace cannot be overstated. Even as abuse of older, illicit drugs such as heroin, cocaine, marijuana, and amphetamines persists, new mind-altering chemicals continue to emerge, contributing to an ever-changing landscape of drug abuse and its physical and psychological consequences. Abuse of multiple drugs, and the combined use of alcohol and illicit and prescription drugs, have become increasingly common. The growing and continually evolving problem of drug abuse demands novel strategies for drug testing that reliably and reproducibly detect drugs of abuse, offer an effective solution to persistent problems related to test accuracy and the potential for sample adulteration and substitution, and speak to the growing concerns over issues of individual privacy. Oral fluid testing is a reliable, new technology that overcomes many of the problems of older methods for drug detection.

Advances in sample collection and drug testing technology are driving the development of new methods for the detection and monitoring of illicit drug use. At the forefront of this technology is oral fluid testing, also referred to as "saliva testing," which is an accurate, effective, and robust testing method. It relies on a simple and straightforward collection process for obtaining an oral fluid sample (composite fluid consisting primarily of saliva mixed with other oral fluids), combined with subsequent sample analysis techniques, which are based on proven drug screening assays that

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Disclosure / Dr. Cone serves as a scientific consultant to OraSure Technologies and has stock ownership in OraSure Technologies.

have been cleared by the U.S. Food and Drug Administration. Oral fluid testing offers a viable alternative to urine testing, the current standard technology to test for drugs of abuse. Oral fluid testing allows for the precise detection and monitoring of all drugs tested for in current urine-based drug testing programs, and, importantly, alleviates the embarrassment associated with urine testing, and with direct observation during sample collection.

Oral fluid testing preserves individual privacy while still allowing for direct observation of sample collection. It therefore minimizes the potential for sample adulteration or substitution, problems that continue to plague urine testing programs. Many private industries have adopted oral fluid testing as their primary means of drug testing, and the Department of Health and Human Services has proposed guidelines for oral fluid testing in federal workplace programs.[1]

Scope of the Problem

Drug abuse is rampant in the United States and spans a broad spectrum of age and societal class. It knows no geographic, racial, or demographic boundaries. A review of emergency department visits for drug overdose in major U.S. cities revealed predominant use of heroin, cocaine, cannabis, and methamphetamine.[2] Even as these drugs retain an active following, though newer drugs continue to arrive on the scene such as Ecstasy — frequently abused in Europe and becoming increasingly popular among adolescents and young adults in the United States — they present a growing and ever-changing challenge for drug testing programs, as users mix and match from an expanding array of illicit agents.

The panoply of drugs of abuse is one of the challenges facing drug testing, which has been a key tool used by the military, government, and private industry as a deterrent of drug use dating back to the mid-1980s. Urine-based drug testing programs have been criticized on two fronts: 1) sample collection performed in a setting that preserves individual privacy leaves open the possibility of sample substitution or adulteration, calling into question the accuracy and reliability of the results, and 2) observed urine collection strategies add to the embarrassment and indignity associated with drug testing, and contribute to the growing concern over invasion of privacy. These two key issues, sample adulteration and invasion of privacy, define the current dilemma in drug testing: how to maintain an effective program while at the same time, respect the privacy of the individual.

The solution must focus on the core problem, which is the collection of the test sample. There exists a collection process that is simple, less invasive, and less embarrassing, yet provides the same assurances of accuracy and reliability as are associated with urine testing programs. New drug testing methodologies, such as oral fluid testing, have emerged that exploit novel technologies and offer alternatives to urine-based drug testing.

Unique Benefits of Oral Fluid Testing

Oral fluid is a viable alternative to urine for drug testing. It is readily accessible for collection and can be obtained under direct observation without risk of invasion of privacy, thus minimizing the potential for sample adulteration or substitution. Furthermore, a significant body of scientific information to support the validity of oral fluid testing has accumulated over the past decade.[3]

DRUG TRANSFER TO ORAL FLUID WILL CONTINUE AS LONG AS THE DRUG REMAINS IN THE BLOODSTREAM; AND, THUS, DRUG DETECTION BY ORAL FLUID TESTING IS AS EFFECTIVE AS URINE TESTING OVER A COMPARABLE TIME PERIOD.

Of significance is the utility of oral fluid for the detection of recent drug use and the ability to detect parent drugs, as well as drug metabolites. In general, drug testing in oral fluid can detect drug use during the previous 24 to 48 hours. Regardless of whether a person swallows, snorts, or smokes a drug, the drug will eventually be absorbed into the bloodstream. As the body begins to rid itself of the foreign substance, the liver and kidneys serve as filters, converting the active drug to less harmful inactive byproducts, eliminating these as waste in the urine. At the same time, the circulating blood delivers portions of the drug to other tissues and organs, including the salivary glands.

Blood flow to the salivary glands is rapid, and drug transfer from the circulation occurs relatively quickly after drug use.[3] Drug transfer to oral fluid will continue as long as the drug remains in the bloodstream; and, thus, drug detection by oral fluid testing is as effective

as urine testing over a comparable time period. The direct relationship between blood levels of a drug and the presence of the drug in oral fluid provides a key advantage in applications that focus on safety-sensitive populations, such as employees involved in transportation or workplace accidents.

Of further significance, a drug detected in oral fluid is typically the active drug component, the substance that causes the psychoactive effects, euphoria, and impairment. In contrast, urine testing typically detects inactive drug metabolites. For example, the body converts heroin to 6-acetylmorphine and morphine, the active drug components that produce the psychoactive effects. Tests on oral fluid can detect these two drug components, whereas urine tests primarily detect inactive morphine metabolites such as morphine-3-glucuronide.

Oral fluid offers distinct advantages for drug testing, and these include the ease and incorruptibility of sample collection. Furthermore, the results of oral fluid testing may have greater relevance to understanding the effects of drug abuse and assessing an individual's behavior, job performance, and safety risk. As the presence of drugs in oral fluid is usually related to the amount of drug in the bloodstream, oral fluid testing may serve as a better, real-time indicator of whether a worker is impaired or unfit for duty. The presence of an active drug in oral fluid demonstrates that the drug was present in the individual's body at the time of sample collection. Therefore, a positive oral fluid test for heroin, for example, is a strong indication that the individual was under the influence of the drug when tested. In contrast, although the effects of heroin typically last for several hours, inactive heroin metabolites may appear in the urine for several days after use.

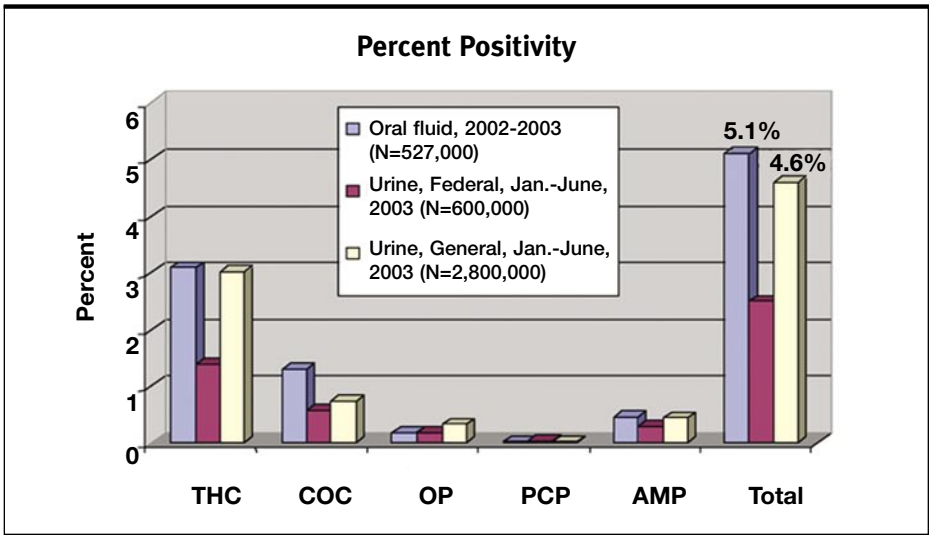

Figure 1. Comparison of positive drug prevalence rate by oral fluid testing (blue) to federally mandated (red) and general workforce (yellow) urine drug testing programs according to Quest Diagnostics' drug testing index. Key: THC, tetrahydrocannabinol (cannabis); COC, cocaine; OP, opiates; PCP, phencyclidine; AMP, amphetamines. (Data supplied by E. Cone, personal communication.)

Minimizing Sample Adulteration and Dilution

One only has to look on the Internet to find thousands of products being promoted for the sole purpose of "beating the urine test." Sadly, interest in these products has spawned a substantial cottage industry. Scientific studies have shown that many of the products sold on the Internet can effectively reverse a positive test result. Chemicals such as strong oxidizers, enzymes, or soaps may interfere with many urine screening tests, and adding these to a urine sample can yield false negative findings. Testing laboratories struggling to keep abreast of the continuing proliferation of new and more effective adulterants are becoming increasingly frustrated and challenged by the growing sophistication of these products. This problem will continue to cast a shadow of uncertainty over urine-based drug testing programs until strict measures are taken to perform sample collection under extremely close supervision — raising, once again, the issue of individual privacy.

Adding to the concern over the potential for adulteration of urine specimens at the time of collection, is the growing awareness among drug abusers that drinking excessive amounts of water offers another easy and relatively effective means of confounding the drug test. Consuming excess fluids can dilute drug concentrations in urine to below reportable levels. In fact, sample dilution and urine adulteration practices may explain why a recently updated survey of more than 500,000 oral fluid tests indicated that oral fluid produced more positive drug tests than did urine testing (**Figure 1**).

Detecting Acidic vs. Basic Drugs

Oral fluid can serve as a "window" into the bloodstream. Oral fluid is a dynamic fluid, and chemical changes

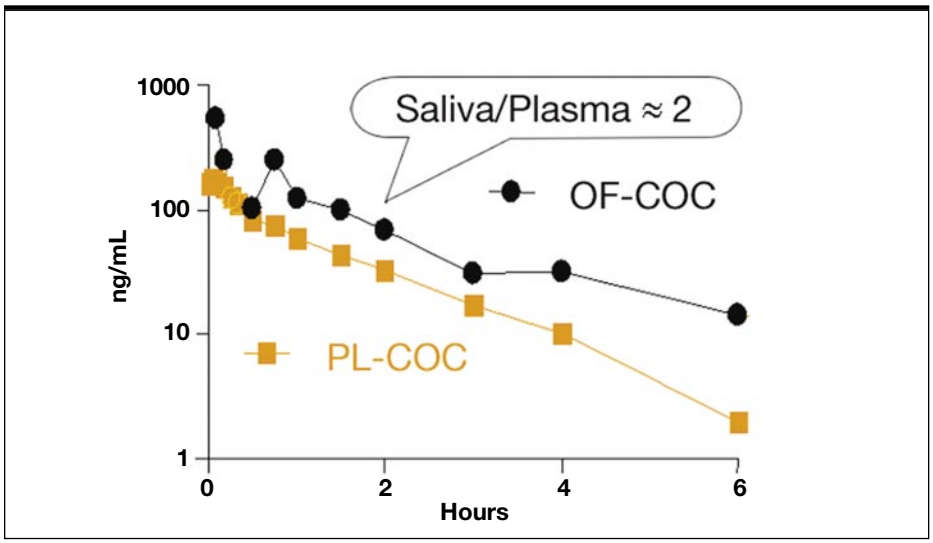

Figure 2. Comparison of cocaine concentrations in oral fluid and plasma of a human subject following a 25 mg intravenous dose of cocaine hydrochloride. Key: OF, oral fluid; PL, plasma; COC, cocaine. (Data supplied by E. Cone, personal communication.)

occur in correlation with changes in flow. As the flow of oral fluid increases, the pH of the fluid rises. The passive diffusion rate of a drug from the blood to the oral fluid is governed by the concentration of the free-drug fraction in the circulation. Drug components cross from the plasma through the epithelial membrane and into oral fluid.

For many of the major drugs of abuse, controlled dosing studies have demonstrated parallel drug/metabolite relationships between oral fluid and plasma.[4] Because of the acidic nature of oral fluids, basic drugs such as opiates, amphetamines, and cocaine tend to be present in relatively higher concentrations in oral fluid compared to blood, thereby enhancing the detection capabilities of oral fluid testing for these drugs (**Figure 2**). In contrast, acidic drug metabolites such as benzoylecgonine, BZE, a metabolite of cocaine, will appear in lower concentrations in oral fluid than in plasma.

Cocaine will tend to leave the basic environment of the plasma (pH=7.4) and accumulate in the more acidic domain of oral fluid (pH=6.0). Cocaine appears in oral fluid within minutes of use and, in general, its presence in oral fluid implies intake within the previous 24-hour period. BZE is the main target analyte for cocaine detection in oral fluid. It has a longer half-life than cocaine and provides a detection window of approximately 36 hours.

Cannabis is the major exception to the oral fluid/blood paradigm previously described. Delta-9-tetrahydrocannabinol, THC, the major psychoactive constituent of cannabis, is deposited directly in the oral mucosa during use, with minimal transfer of the drug from blood to oral fluid because most of the THC in the blood is bound to protein. The window of detection for cannabis depends on the amount of drug used and the frequency of use. Typically, THC can be detected in oral fluid for 12 to 24 hours after use. THC will be present in greatly increased concentrations in oral fluid compared to plasma within the first several minutes of use, but by about 30 minutes, oral fluid levels of THC typically mirror those in the bloodstream.

Passive exposure to cannabis smoke, by virtue of an individual being near a cannabis smoker, can result in transient levels of THC in oral fluid. Time course studies have demonstrated that the risk for THC in oral fluid following passive exposure is minimal, even in a confined space such as an unventilated motor vehicle.[5] Oral fluid levels of THC are relatively low following passive exposure and, with the exception of extreme conditions, passive exposure to cannabis smoke is not a credible explanation for a positive oral fluid test.

How Does Oral Fluid Testing Work?

Obtaining an oral fluid sample appropriate for drug testing is relatively easy when one uses a well-designed collection device. An oral fluid collector is typically comprised of a small absorbent pad mounted on a plastic stick, similar in size to a small lollipop. An individual simply holds the stick and inserts the collector into the mouth, usually between the cheek and gums. Within two to three minutes, the pad will absorb all the oral fluid it can hold. The individual then removes the collector and typically places it in a sample vial, sealing the vial for transport to a testing laboratory.

From this point on, testing of oral fluid is very similar to urine testing. The oral fluid specimen is placed in a sealed container and shipped to a laboratory, together with a chain-of-custody form. Laboratory testing procedures initially begin with a screen-

ing assay that eliminates negative, drug-free specimens. No further testing is needed for negative samples and the results are reported to the authorizing agent who requested the test. Specimens showing evidence of being positive for drug in the screening assay are then retested using a sophisticated, highly sensitive confirmation assay that can accurately determine drug content. The specific drug or metabolite present is measured and, if the amount is sufficient to meet reporting criteria, the result is reported as positive. Both negative and positive results are sent to the authorizing agent, usually within 24 to 48 hours after receipt of the specimen in the laboratory.

Collection of oral fluid is easily conducted under direct, close observation. Collection procedures should allow for a short waiting period, usually about 10 minutes, where the individual has had nothing to eat or drink, followed by the observed collection. This allows virtually no opportunity for adulteration or substitution of the specimen. Furthermore, with regard to the problem of dilution of urine samples, drinking excess liquids prior to reporting for oral drug testing does not alter drug concentrations in oral fluid samples.

Conclusions

The advantages and proven effectiveness of oral fluid testing in the workplace have led many drug testing programs to adopt this new technology in place of urine testing. Initial reactions suggest that employees are comfortable and pleased with the simplicity and dignity of sample collection. Treatment specialists in drug rehabilitation programs view oral fluid as a promising new technology that may allow for a "therapeutic approach" to monitoring drug treatment. Additionally, police and law enforcement officers are looking to oral fluid testing as a possible means of on-site testing for active drug levels without the requisite requirements for more invasive procedures. The Department of Health and Human Services is currently drafting guidelines for the use of oral fluid as a specimen for testing in federal workplace testing programs. Expanded use of oral fluid testing in the federal workplace and other settings will set an example for the widespread adoption of oral fluid as a viable, convenient, and broadly accepted alternative to urine-based drug testing.

In the future, new technology currently in development will enable point-of-collection oral fluid testing, adding to the advantages that make oral fluid the specimen of choice for on-site drug testing. With this emerging technology, samples can be tested immediately after collection, yielding instantaneous results, with no further testing required following a negative test result. Specimens that test positive would still require confirmatory laboratory testing. The obvious benefits of point-of-collection oral fluid testing for drugs of abuse would include the considerable time saved, as negative tests account for the large majority of tests performed, as well as the ability to identify potential drug users earlier in the process, before their impaired judgment or performance can result in serious consequences. Currently available oral fluid tests and point-of-collection test kits now in development are revolutionizing drug testing, improving ease of use and reliability, while offering the same accuracy and precision across a broad spectrum of drugs of abuse as traditional urine-based testing. CDA

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Cultural, Behavioral, Social, and Psychological Perceptions of Saliva: Relevance to Clinical Diagnostics

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ABSTRACT

The search for a resource that can be used to detect a broad range of diseases easily and reliably is akin to a search for the diagnostic Holy Grail. Yet, each of us may have inside our mouths, a key to the pathological and disease biomarker library hidden inside our bodies. Saliva — the source of all this information — is the secretory product of glands located in or around the oral cavity. If one could read the stories of diagnostic information present within saliva, then the abundance of information waiting to be found could be comparable to a vast vault of information, such as the Internet. Upon dissection of this data, it would be seen that the source of this information is from saliva's origin as a filtrate of blood, and that the validity of both mediums should be equal. Although one day this may be the view, most people's hold of saliva, current and past cultures, have fared much more diverse meanings to the secretion. Ivan Pavlov's experiments has shown how closely tied salivation is with the thought of food, one of life's primary indulgences. The relationship between salivation and behaviors within our daily lives is undeniable. Yet most people never appreciate the uniqueness of saliva. Throughout the world, saliva carries definite positive and negative connotations, based upon its social, psychological, behavioral, and cultural settings. The thought of saliva may be viewed as grotesque in one population, yet may be the vehicle of blessing in other cultures. Saliva's double nature brings up some interesting cultural, social, behavioral, and psychological points about how saliva is perceived in the world, some of which are subsequently stated in order to present saliva as the spirited fluid it is.

Some of the history for saliva's negative views stem from the discovery that saliva may carry airborne pathogens and respiratory diseases. This stemmed from various events throughout history around the world. Early in the mid-19th century, spitting in public was commonplace, usually associated with chewing tobacco. The turn from its socially acceptable position toward being shunned started in the mid-19th century, when human saliva was placed under the light microscope and was found to contain microorganisms and germs. The surge in placing saliva in a negative light continued during the late 19th century when the famous German physician

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Figure 1. Infant salivating, a lovable sight to a parent.

Figure 2. South America tradition of "chicha" using the digestive and fermentative properties of saliva.

Figure 3. The "Evil Eye," the source of misfortune in Greek culture. Spitting is an effective way to ward off the evil eye.

Robert Koch discovered samples of sputum was found to harbor *Mycobacterium tuberculosis*, the deadly bacteria responsible for the widely spread disease of tuberculosis at the time.[1] Influenza and other respiratory pathogens soon were discovered to also be transmissible through respiratory droplets originating from infected individuals. Although saliva may, in fact, be the medium by which some diseases spread, it is merely an innocent vehicle. The phlegm and mucous from the respiratory tract is the original source of bacteria and viruses. It is arguable that saliva is not the problem; sputum is the true culprit and so saliva should not be automatically tied with germs and disease.

Social

Although ~1.5 liters of saliva is being produced by the salivary glands into our oral cavity daily, salivation, with the rare exception of infants and toddlers (**Figure 1**), is often perceived as socially inappropriate or even unacceptable. In America, accepted practices of spitting are usually reserved for sports athletes. In baseball, America's favorite pastime, spitting is a common practice in the dugout or on the field. Although commonly associated with chewing tobacco, the act is also a gesture of machismo, and even by players who are not chewing. Pitchers spit on the mound; batters spit onto their hands, all in attempt to gain good luck. Football players are viewed as some of the toughest athletes in any sport, so it would not be uncommon to view them spitting on the field during a heated and competitive match. Basketball players such as the legendary Boston Celtics Larry Bird, is often remembered for spitting on his hands for good luck at the beginning of every game. Spitting is present even in the exclusive world of golf, where there are superstitions to spit over a bridge or onto one's shoes for good fortune.

Spitting in public, however, is seen as rude and, sometimes, offensive. The expectoration of saliva is generally not seen as being politically correct. In a motion of disrespect, spitting may also be used as the popular choice for an offensive gesture. Sayings such as "I spit on you" or "I spit on your grave" precisely convey those connotations. In the Mexican culture, "spitting on a parent" is a form of elder mistreatment, a form of family violence. However, in other cultures, saliva is much more intimately tied with common beliefs and rituals.

In South America, a tradition that originated with the Incans persists today. Women in Peru, Bolivia, Venezuela, and countries along the Andes continue the process of fermenting manioc root, maize, or fruits into the intoxicating alcoholic "chicha," using the digestive and fermentative properties of their own saliva (**Figure 2**). It is believed the amylolytic enzymes present in saliva convert starches within the plants into fermentable sugars. In fact, the name chicha is derived from the Spanish word "chical," which is loosely translated as "to spit" or "saliva."[2]

Hundreds of years ago, the drink was of great importance to the Incans. South Americans today still view it as an essential part of their culture. The Incans widely used the drink during their rituals, and chicha eventually became as valuable as gold itself as the drink was commonly sold as a key economic currency within their society. Today, chicha is still used among the Amazonians as a staple within their diets, as well as a work incentive and social drink. Although there are more modern ways to ferment the maize or other products, it is commonly believed the unique sour taste of chicha is lost if no contact with human saliva is made, a property that may be conferred from the presence of *Lactobacillus* bacteria within the sputum of donating women.

Ritual

The Greeks also have had longstanding traditions with saliva and the act of spitting. Generally, Greeks will

spit to ward off bad luck in hopes of good luck and well-being. The source of misfortune is usually believed to originate from the "evil eye," thought to be a shadowing entity of bad luck and evil (**Figure 3**). It is believed that two ways of effectively warding off the evil eye are large quantities of garlic, or the more commonly practiced gestures of spitting. Spitting is commonly seen during ceremonious events. The source of this gesture is thought to be within the Greek Orthodox Church and the blessing of the Holy Trinity. During the ceremony of baptism, the priest will usually bless a child with holy water, after which he will spit three times on the ground to represent the Holy Trinity in renouncing the devil. This blessing is also carried on during Greek weddings, where the guests will gingerly spit on the bride to project their blessings and good fortune. Spitting also has its place in everyday life. Greek fisherman will commonly spit onto their nets before hoisting them over the bows of the ship to ward off any evil that may prevent them from catching a bountiful yield. The popular response to a compliment may be three spits to the ground. It is also common in Greek culture to make three spitting gestures onto their clothing and onto each other during greetings in order to ward off the infamous evil eye. Thus, the act of spitting is widely present in proper Greek etiquette.

Spitting

It is customary for Greeks to spit as a way to ward off evil, and particularly the evil eye (mati). It is not the typical sportsman spitting; it is very subtle and hardly any spit comes out. Greeks are often seen uttering "ptousou" when they feel that the evil eye could dawn upon something.

For instance, when attending a Greek wedding, the focus is usually on the bride. Everyone is looking at her, some with envy, which could possibly provoke the evil eye. Greeks usually spit and say "na me se matiasou," which generally means one will not cause the evil eye to dawn on the bride. This is also common when buying a new car or house. Greeks spit to take off the evil eye that could be caused by jealousy (evil).

From: http://www.europeans.co.za/cafeneio/superstitions.htm

Health and Medicine

Nowhere is saliva more integrated within celebratory rituals and as a form of alternative medicine than in various tribes of Africa. Different tribes may use saliva, pure or mixed, with traditional medicines, in a minor part of their healing treatment, where others may use saliva in large contribution, regarding it as the cure-all for most ailments. For example, the Somali use saliva as the primary remedy for an open blister.[3] To alleviate the pain and swelling of snakebites, the Somali usually treat the ailment with mixed butter and saliva.[4] Within other tribes, such as the Azande of Sudan and the Masai of East Africa, saliva may be used as a first-aid astringent for minor wounds, alone or mixed with herbal plants, respectively.[5] The Bena of Tanzania use saliva to treat boils, and mothers of the Masai tribe use it to treat insect bites and swelling.[6] The primitive, yet abundant, source of saliva makes it an easy medicinal agent for common ailments.

In other parts of Africa, saliva carries a more spiritual meaning. It is believed within the Wolof tribesmen that saliva may confer blessings and curing properties from the source to the target.[7] The theory behind this parallels the beliefs of homeopathy, in which water is able to retain the memory and essence of its source. Therefore, following the birth of a child, it is common practice to invite the newborn's elders to bless him or her with their salivary secretion. The female elder graciously spits on the newborn's face and the male elder spits into the ear and rub his saliva across the infant's face, as a welcome into the world.[8] Similar rituals of direct salivary inoculations occur within the people of Ashanti in Ghana, where the spiritual enlightenment of an infant is brought about through the grandfather spitting into the mouth of the newborn.[9] Saliva is not viewed as a medium for disease transmission, but instead as a mode of passing on spiritually, curing properties, and life.

In Asia, the act of spitting may date back to 4,000 years ago when the betel nut (an areca nut, betel leaf, and lime mixture) was habitually chewed in Thailand, India, Philippines, Taiwan, and Indonesia. This legal stimulant is likened to chewing tobacco. The exotic drug releases plant alkaloids, which are readily absorbed through the mucous membranes of the mouth, causing excessive secretions of saliva.[10] This effect is clearly visible as the saliva is reddish-brown in color. Spitting is recommended since swallowing can produce an uncomfortable burning sensation. Betel nut chewers find the drug pleasurable, aromatic, and cleansing. The streets of Taiwan and India are noticeably stained with reddish splotches from this habit. Besides in the practice of betel nut chewing, the act of spitting is a traditionally accepted practice in Asia, regarded as an appropriate component of good personal hygiene. In large cities with severe air pollution, coughing and spitting is widespread due to the need to remove phlegm and clear the throat.

More recently, however, much is

being done to decrease the act of spitting. After the severe acute respiratory syndrome outbreak in 2003, China sent 1,000 sanitary workers to Guangzhou City to monitor public spitting to prevent further spread of SARS. China, Hong Kong, and Taiwan have since launched many campaigns to discourage the "filthy" act by issuing fines and summons to any public spitters. The desire to reduce this practice on public streets has resulted in strict government fines of up to $5,000 in Singapore[11] (**Figure 4**). Whether or not these fines are warranted remains arguable, but the shift from spitting as a natural part of life toward a more serious link with death is the current trend.

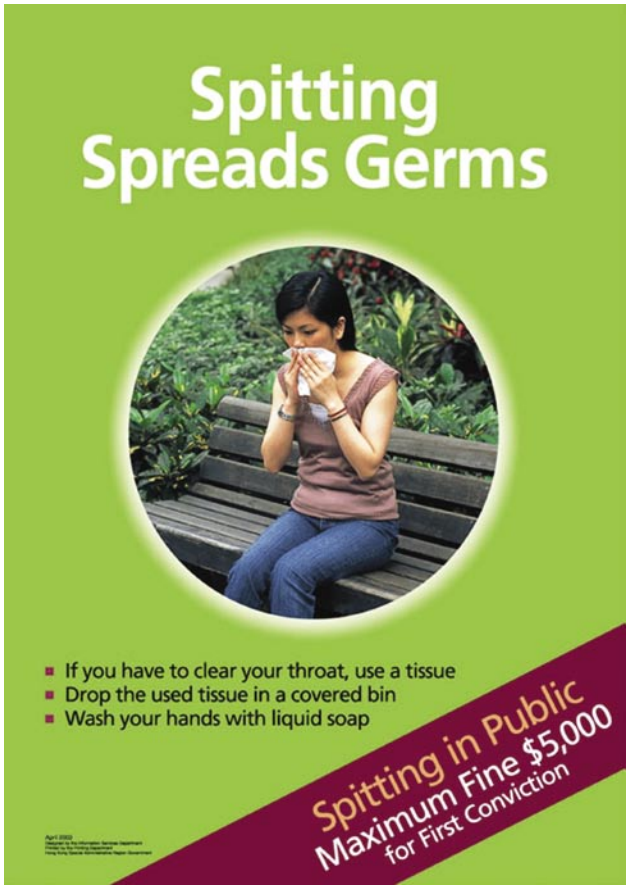

Figure 4. "Don't spit" fliers in Asian countries, (courtesy of the Hong Kong Special Administrative Region Government).

Psychological Aspects

Darwin's Theory

In any culture, a kiss is seen as a heavenly gentle gesture, shared during the most intimate of settings. Yet, in order to appreciate the joys of kissing, one must inevitably make contact with the other person's saliva. People are generally willing to undergo the act with a loved one, yet are more reserved about the thought of kissing a stranger. Pet owners may allow their own pets to lick them, yet the affection of other pets may be rejected. A certain amount of trust or an intimate bond between two entities is required to get over the idea of sharing saliva with another being.

Throughout the history of mankind, this selective nature may have evolved as a method of protecting oneself from being exposed to the many potential diseases carried within saliva. However, the persistence of the act must hint toward the presence of positive benefits as well. Perhaps, kissing not only spreads noxious bacteria to the organism, but also bacteria that may actually be beneficial to it. In nature, it is widely observed in various animals that a mother will inoculate her offspring by covering them with her own saliva within the first few hours of birth. This ensures the passing of vital bacteria and antibodies that will assist in the offspring's digestive and immune functions without which may lead to a loss of certain immune functions. Saliva may play an additional role during infancy by having a large influence on the development of an individual's future social behaviors. Scientists have observed that among Mongolian gerbils, cues within saliva play a key factor in deciding the response behaviors between the young and the old, parents and offspring, and males and females.[12]

Perception

Although most people do not have a problem sharing saliva during kissing, there is a much more conservative view in sharing inanimate objects associated with saliva contact. A toothbrush, a spoon, and even a straw are all objects that are taboo to share, simply because contact has been made with someone else's saliva. The psychological disconnection between saliva inside the mouth, and saliva exposed to the outer environment may be due to the way our mind sees a difference between saliva that has passed beyond the mouth and saliva that remains within the confines of our bodies. Dr. Gordon Allport, a social psychologist from Harvard, depicted a scenario in

his book, where if one were asked to drink from a cup that had previously been filled with saliva from the same individual, how hesitant would one be to drink his or her own saliva.[13] Allport hypothesized that although the saliva came from the same person, the saliva became nonself and alien to the mind the moment it exited the mouth. This modification in perception may be the leading factor in why people may have difficulties adjusting to the fact that saliva has true and viable diagnostic information about the internal body from which it came.

Science and the Future

When evaluating saliva on a molecular level, saliva contains mostly water. It is fitting that saliva is predominantly composed of the essence of life, since saliva is the vehicle for so many things relating to it. Saliva is also composed of various constituents, which confer numerous properties to the rich medium, many of which are crucial in everyday acts of living. Among the top functions of saliva include antibacterial roles through peroxidases, mucins, and cystatins; buffering roles using carbonic anhydrases and histatins; digestive functions using amylases, mucins, and lipases; and the more obvious role of lubrication through mucins and statherins.

The importance of this heterogeneous mixture cannot be understated. Yet, the robust duties of saliva are commonly taken for granted and only truly appreciated when the precious medium is not found in abundance within the mouth, as in radiation or oral cancer patients. It is within these individuals where speech and severe eating difficulties routinely surface. It becomes extremely difficult for patients with minimal salivary flow to eat normal everyday types of food, such as bread, meat, and other solid foods due to the difficulties in chewing and swallowing.[14]

Along with sacrificing some of the comforts of life, functional pathologies within the oral cavity also quickly begin to emerge. Cavities are much more prevalent in patients with lowered salivary flow due to the loss of salivary bathing of the teeth, which normally confers a buffering role and antibacterial medium. Fittingly, Frank Oppenheim, chairman of the department of periodontology and oral biology at Boston University, summarized the importance of saliva's constituents with the statement, "If saliva were (merely) water, we would have little stumps of teeth or no teeth at all by age 20 — we would have dissolved our teeth away."[15]

The functional value of saliva has long been thought to outweigh the diagnostic possibilities. More recently, however, the evidence for using saliva as an accurate diagnostic tool in diseases such as HIV, various forms of cancer, diabetes, arthritis, and heart disease show there is much more information in saliva than previously thought. With the abundance of information that may be contained within, saliva might possibly play an even greater role in the daily lives of people.

Scientists are slowly transitioning from viewing saliva as a diagnostic outcast in comparison with blood or urine, and are starting to view saliva as an abundant valuable resource. The advantages of using saliva testing as a diagnostic tool are due to its noninvasive nature, in addition to the quick and reliable results.[16] The current trend in the psychology of scientists is leaning toward saliva being seen in a positive light, with the potential for extracting data higher than ever.

However, there may be cultural perceptions that form barriers against that which professionals already are beginning to discover, and those will slowly be overcome inevitably with time. Four articles in this issue of the *Journal of the California Dental Association* highlight the exciting science that is emerging from the utilization of saliva as clinical diagnostic fluids. The gap between saliva and other disease diagnostic biomedia (blood, urine, cerebral spinal fluid, tear, nipple aspirate, fecal matter) is rapidly closing. This is primarily due to the rapidity of the emerging sciences, sparked by the initiatives from the National Institute of Dental and Craniofacial Research. Scientific data to benchmark the diagnostic value of saliva against other biomedia will be necessary to assess the disease discriminatory value of saliva. It may well turn out that, similar to the recent finding that saliva is more accurate than blood for oral cancer detection, saliva diagnostics will outperform other biomedia for other disease diagnostics as well.[17,18] This is the quest.

Summary

In summary, it is clear that saliva, due to its readily available nature and noninvasiveness, has historically been associated with a Dr. Jekyll-Mr. Hyde personality. However, when one closely and carefully examines the undesirable associations of saliva, one often concludes that links are largely mythical in origin and/or unscientific. The positive values of saliva, on the other hand, are scientifically based and continue to emerge. If the scientific values and diagnostic utilities of saliva is as good as or better than other bodily biomedia, it will be clear that its ease of obtainment, total noninvasiveness, ease, and pleasantness of use compared to other biomedia (blood, urine, cerebral spinal fluid, tear, nipple aspirate, fecal matter) will and should eventually place saliva as the biomedia of choice in clinical diagnostics. The day when saliva is considered a diagnostically diverse and charismatic fluid should not be far away. CDA

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ture until the area hemorrhages freely.

Sometimes a sock stuffed into the victim's mouth helps reduce distractions. The rescuer, assuming there is one and the bitten person is experiencing syncope by this time, places his mouth over the puncture/slice wounds and sucks out the snake's venom, being careful not to swallow it. It is then discreetly expectorated in a downwind fashion as approved by the EPA. By this time, the snake, not being of a poisonous variety in the first place, has laughed itself to death and is no longer a threat.

I understand this technique is no longer in common use. Even 12-year-old boys not subject to the civilizing influences of society found this procedure disquieting, so it has been supplanted with a more modern treatment wherein the offending snake is counseled and given a severe reprimand. The victim may or may not be covered by his HMO at the discretion of his primary care provider.

Too late for me, however. My antipathy toward snakes is too deeply rooted to be influenced by herpetologists' unconvincing explanations of their gentleness and general benefit to the ecology.

In my view, ***every*** snake is a flexible, protein-based tube of neurotoxins. Its one purpose in life is to propel itself straight for my jugular where the tourniquet/Scout knife technique is not applicable. The fact that I have not seen a snake for upward of 30 years is no excuse to relax my vigilance.

All of which explains why I was visibly shaken when my granddaughter announced that if we expected her to emerge from her high school biology class with anything more than a C-, it would be expedient to purchase a snake for her and a receptacle to contain it. What role the snake would play in the furtherance of her education was not clear, but its procurement was not to be denied.

There are actually reptile stores, up-

The snake and I maintained our distance and regarded each other with mutual loathing.

scale boutiques where exotic vertebrates are offered to reptilian aficionados at equally exotic prices. My granddaughter and I peered gingerly at a colorful variety of snakes, lizards, chameleons, and turtles. The captive denizens stared back, transfixed as if fashioned of stone.

"Here's a nice corn snake," said the pony-tailed, eyebrow-pierced youth serving us. The snake is about 18 inches long, banded orange and white. I conceded that it might be considered as attractive as a four-alarm fire silhouetted against an evening sky enthralling an arsonist. Another snake of the opposite gender might even offer a judgment of "Hubba, Hubba!" The snake and I maintained our distance and regarded each other with mutual loathing.

My dislike for snakes is scientifically based on these factors: No. 1: Locomotion. The verb "slither" had to be coined for snakes. Should it ever become necessary for you to inspect the underside of a snake, even though common sense dictates otherwise, you'll notice it has no legs or feet. If it were human, it would be a quadriplegic and could park in restricted zones. In spite of this handicap, a really fast snake on Full Red Alert has been clocked at 8 mph. I realize this doesn't seem too impressive compared to the human he was chasing who was hitting 52 mph on the straightaways.

The slithering is accomplished by one of four methods: The Undulating Crawl or Serpentine, the Caterpillar or Rectilinear, the Sidewinder, and the Concertina. All four methods of locomotion are unnatural, if not actually obscene, and I don't want to talk about them any more.

Reason No. 2 why snakes and I are not pals is that they are inarticulate. They do not bark, moo, meow, chirp, or quack. Compared to a snake, a mime is a regular chatterbox. You can't call, "Here, boy! C'mon let's go for a slither." No. They hiss. They stare at you with those slit eyes, flick that forked tongue and they hiss. I cannot be simpatico with anything that hisses and slithers. Or scuttles. A forthright animal worthy of trust does not scuttle.

Assuming that the snake, in order to survive even one semester of biology, must eat *something*, I questioned the Snake Man about the dietary requirements of our purchase. I figure a corn snake eats corn, right? Wrong. "Mice. He eats mice," he said.

"Well, that's unfortunate, we don't have any mice. Let's go," I whispered to my granddaughter.

"Not a problem," interrupted the Snake Man. "We have plenty of mice right here." He indicated a cage where dozens of tiny mice, hairless, sightless and unsuspecting, stumbled around in sweet rodent innocence.

Out of respect for your sensibilities, I will spare you the gruesome details, but take my word, there is no sight more hurtful to the human psyche than witnessing a snake devouring a live mouse. Forever vivid in my memory is the vision of the unhinged jaw, the slow, peristaltic bulge moving tailward, the mouse's tail still signaling fruitlessly as it disappears.

It's the stuff of nightmares and the orgy repeats every week as long as the snake is our responsibility. We are petitioning the guidance counselor for a transfer to Early American Folk Dancing.

FREE TO GOOD HOME: Corn snake, like new. Lo miles; ideal pet; loves children and mice. Easily trained to slither and hiss on command. Complete with cage and subscription to "Rodent Raising for Fun and Profit." Call anytime, day or night — will deliver; 555-1212. CDA

Robert E. Horseman, DDS

Snakes: The Stuff of Nightmaresssssssss

Call me prejudiced. Call me paranoid, biased and ignorant if you like, especially if you are larger than I am, but the fact of the matter is, *I don't like snakes*. This reptilian anathema goes back to the early days of my Boy Scout career. Prior to my induction into the BSA, I considered snakes to be just overachieving worms, just as a rat was a buff mouse. But as every Boy Scout worthy of his Tenderfoot badge soon learns, snakes present such a life-threatening hazard that an entire section in the BSA Handbook is devoted to coping with anticipated encounters with them.

By this time, the snake, not being of a poisonous variety in the first place, has laughed itself to death and is no longer a threat.

Our motto "Be Prepared" was not a hollow challenge. We had our tourniquet (neckerchief) and our Boy Scout knife (precursor of the Swiss Army knife) with its leatherpunch for punching leather and its main blade, so dull from playing mumblypeg and carving trees that it couldn't slice margarine. Armed with this snakebite armamentarium, our instructions were clear: The moment one of the 42 million species of snakes bites you or a friend, apply the tourniquet between the bite and the victim's heart. The handbook assumes the snake has had the decency to not go for a midsection or butt bite. Tighten until the extremity turns indigo, then grasping the Scout knife firmly, slice an "X" over each fang punc-

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